LOUVAIN DRUG RESEARCH INSTITUTE

REPORT 2015

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Marie-Paule Mingeot-Leclercq

Vice-presidents:
Patrice D. Cani
Raphaël Frédérick
Bernard Gallez

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http://www.uclouvain.be/en-ldri.html

UCL Université catholique de Louvain

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PREFACE

The general objective of the **Louvain Drug Research Institute (LDRI)** is to develop cutting-edge research, fundamental and/or applied research, in the field of drugs. Overall research activities are developed from target identification & validation to clinical practice through hit identification/hit to lead, preclinical evaluation, pharmaceutics, clinical assays and optimization of clinical practice.

The LDRI is located on the Health Sciences Campus of the *Université catholique de Louvain* (UCLouvain) in Brussels. The University Hospital (*Cliniques Universitaires St Luc*) is located within walking distance of the Institute. Around 100 researchers presently work in the Institute.

Since its creation 6 years ago, the LDRI increased the external visibility of its research activities, and increased its autonomy to adapt the resources to research priorities and needs. Research excellence conducted at the LDRI has led to an increase both in the number and in the quality of the publications in well recognized international journals. According to the prestigious QS World University Ranking, our research activities in Pharmacy and Pharmacology are recognized in the Top 50-100 Universities over the world, UCLouvain being ranked as the first French-speaking University in Belgium, and the second at the national level after the KULeuven.

"Bridging sciences for better health" is the LDRI's motto. The LDRI is proud of the diversity and wealth of its research despite its relatively small size, and the limited number of senior researchers who are also involved in teaching and institutional activities. The members of the LDRI join their forces to form a multidisciplinary Institute where all major aspects of the drug are covered. The research activities range from the design or identification of a new drug (and the discovery of new targets) to its optimal use through all modern means of evaluation. The approaches use in vitro (membranes and cells) and in vivo pre-clinical models (small animals). Patients-oriented research is focused on the pharmacokinetics/pharmacogenomics and clinical pharmacy.

The LDRI is organized in seven functional research groups led by highly motivated academics who have the responsibility to advance their own research fields. The research groups are closely linked by a series of common research projects, and they share large scientific equipments. The Institute is also supported by two technology platforms that gather experts and outstanding equipments in innovative technologies (Mass Spectrometry and Magnetic Resonance). Most groups in LDRI are participating in interdisciplinary research projects with other UCLouvain Institutes (within the Health Sector or with other Science and Technology Sector such as Institute of Life Science, and Institute of Condensed Matter and Nanosciences), or with other Universities both nationally and internationally or industries.

The LDRI is also a wealthy niche for the education of young researchers. To stimulate continuously the interdisciplinary approach, research seminars are organized weekly with an alternation of presentation by senior researchers generally coming from other institutions, and data-club presented by young researchers enrolled in the Doctoral School in Pharmaceutical Sciences. We are also happy to have a continuous influx of doctoral students and post-docs with a large proportion of foreign scientists.

In addition to training young researchers, the LDRI ensures the continuous dissemination of knowledge to the scientific community, and offers expertise for the authorities of public health and/or pharmaceutical, chemical and biotechnologies industries.

Thanks to our internationally competitive research, our involvement in creating and fostering new knowledge with a direct impact in healthcare, and our ambition to develop efficient partnerships with industry and society, the LDRI activities closely meet the ambition of the League of European Research Universities.

In the first part of this report, a brief overview is presented concerning our objectives and mission statements, research groups, decisions making and management, human resources, funding, and scientific output of the LDRI as a whole. Next, the different research activities led by the different research groups are described.

We hope that this report will enrich the visibility of the Louvain Drug Research Institute. Enjoy the reading!

Marie-Paule Mingeot-Leclercq,

President of the LDRI

Patrice D. Cani, Bernard Gallez and Raphaël Frédérick,

Vice-Presidents of the LDRI

LDRI – 2015 HIGHLIGHTS

The next pages summarize the major highlights of 2015.

InBev-Baillet Latour Grant for Medical Research 2015

InBev-Baillet Latour Grant for Medical Research pertains to one of the topics of the international "InBev-BAILLET LATOUR" Health Prize. The latter was established in 1977 to be awarded periodically to recognize the merits of a person whose work has contributed prominently to the improvement of human health in the fields of metabolic disorders, infectious diseases, neurological diseases, cancer and cardiovascular disease.

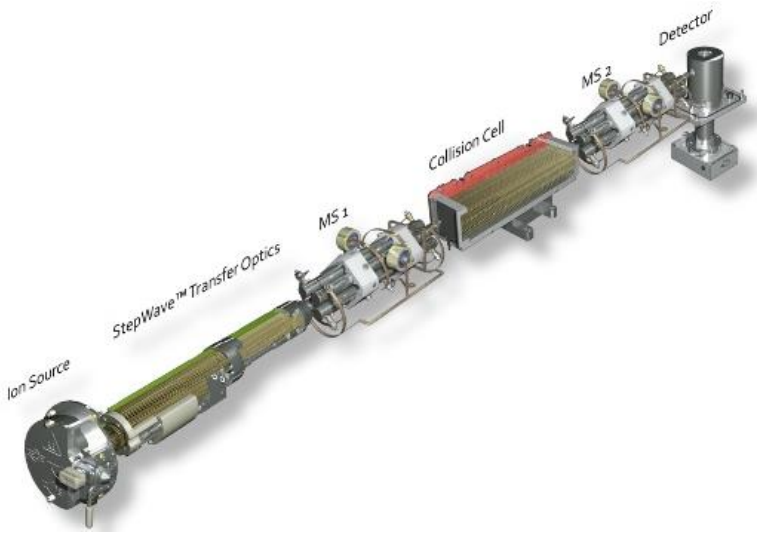
In 2015, **Professor P. Cani (LDRI)** received the InBev-Baillet Latour Grant for Medical Research, as young independent investigator to recognize outstanding scientific achievements in the field of metabolic disorders for the benefit of human health and to encourage the laureate in the pursuit of his career.

Equipments

Outstanding equipment will be installed in the beginning of 2016, increasing the innovative technologies already in place at LDRI.

A new UPLC-MS for the MASSMET platform

Thanks to a FRS-FNRS budget, and with the support of the Health Sector, we were able to buy a new **UPLC-tandem quadrupole** that will complement the HPLC-LTQ-Orbitrap of the platform.
While numerous compounds can be easily quantified by HPLC-UV or GC-FID methods, including from complex matrices such as tissue or plants, the sensitivity of these methods can be too low to reliably quantify low level endogenous metabolites or drug metabolites. While the orbitrap gives access to the exact mass and therefore helps determine a compound's structure, the tandem quadrupole, with its higher sensitivity, will be very helpful in the quantitative studies.
A tandem quadrupole is made of two quadrupole mass analyzers separated by a collision cell. This technology, coupled to innovative ion transfer optics allows for high sensitivity.
This novel equipment will be very helpful for many research projects within the LDRI and beyond.



SECTION I – LDRI GENERAL PRESENTATION

I. Objectives and mission statements
II. Research fields and groups
III. Decision-making and management
IV. Human resources
V. Fundings
VI. Scientific output (publications, PhD Thesis)

I. Objectives and mission statements

The general objective of the Louvain Drug Research Institute (LDRI) is to develop and maintain fundamental and / or applied research projects in the field of drugs within the Health sciences sector of the Université catholique de Louvain.

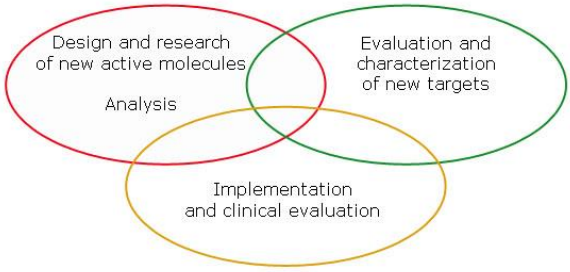
The research axes encompass the discovery and the conception of new active molecules, the study of their pharmacological profile, their metabolism and toxicity, their formulation, and the optimization of their use. These research projects are supported by two technological platforms: mass spectrometry analyses and pre-clinical magnetic resonance.

Research Excellence conducted at the Louvain Drug Research Institute must ensure the following:

- Publications in well recognized international journals and / or patents,
- Training of young researchers,
- Dissemination of knowledge to the scientific community,
- Expertise for public authorities health and/or pharmaceutical, chemical and biotechnological industries.

II. Research fields and groups

Overall, research activities are developed around three poles that range from the design of drugs (and the discovery of new targets) to their optimal use through all modern means of evaluation.



- Design and research of new active molecules pole includes 2 research groups: Medicinal Chemistry (CMFA; rational based-synthesis of new compounds) and Pharmacognosy (GNOS; extraction and identification of new active principles from plants). These groups work in close collaboration with the research group focused on Bioanalysis and Pharmacology of Bioactive Lipids (BPBL), mainly for optimization of analytical procedures.

- Research focused on evaluation and characterisation of new targets are performed by two research groups: (i) Cellular and Molecular Pharmacology (FACM; pharmacology of anti-infective agents [mainly antibiotics], now joining two other research groups involved in clinical research (PMGK and CLIP) to conduct translational research (TFAR), and (ii) Metabolism and Nutrition (MNUT; integrative physiology, metabolism and nutritional approaches) including Toxicology and Cancer Biology (GTOX; mechanisms leading to cell death).

- Finally, implementation and clinical evaluation research are covered by work performed by (i) Population Pharmacokinetics and Pharmacometrics (TFAR/PMGK), (ii) Advanced Drug Delivery and Biomaterials (ADDB; use of drug delivery systems and biomaterials as a means to improve therapeutic outcomes of drugs, (iii) Biomedical Magnetic Resonance (REMA; development of innovative tools using magnetic resonance with applications

mainly in oncology) and (iv) Clinical Pharmacy (TFAR/CLIP; evaluation of the quality of use in medicine and clinical practice) research groups.

All major aspects of the drug are covered from its design to its use.
The approaches use in vitro (membranes and cells) and in vivo models (small animals). Patient-oriented research is focused on pharmacokinetics and clinical pharmacy.

III. Decision-making and management

The LDRI Management Committee is currently composed of a President (Marie-Paule Mingeot-Leclercq) and three Vice-Presidents (Patrice D. Cani, Raphaël Frédérick and Bernard Gallez) elected by the LDRI Council.

The Bureau and the Council ensure the proper functioning of the Institute and are responsible for all major decisions concerning the LDRI.

The Bureau is composed of the President and Vice-Presidents of the Institute and representatives of the temporary scientific (2), administrative and technical staff (2) and academic (2) elected by their peers.

The Council is composed of the permanent scientific and academic LDRI members and representatives of the scientific (3), administrative and technical staff (2). It elects the President and three Vice-Presidents.

An International Scientific Council which has been enlarged to five members provides advices on the research and recruitement strategy. Members are C. Hughes (Queen's University, Belfast), D. Crommelin (Dutch Top Institute Pharma, Leiden; University of Utrecht), J.L. Veuthey (Université de Genève), E. De Clercq (REGA Institute, KUL Leuven) and B. Staels (Université de Lille / Institut Pasteur Lille).


Prof. C. Hughes (UK)


Prof. D. Crommelin (NL)


Prof. J.L. Veuthey (CH)


Prof. E. De Clercq (BE)


Prof. B. Staels (FR)

IV. Human Resources[1]

The total staff of the LDRI in 2015 counted to 104.5 EFT.

Academic staff:

Due mainly to teaching activities and part-time contracts, the number of academics does not reflect the EFT number dedicated to research. Professors paid by UCL spend 75% of their time for research. Among the 12.8 EFT working at the LDRI, 5,6 EFT senior researchers are paid by FNRS and are included in the academic staff.

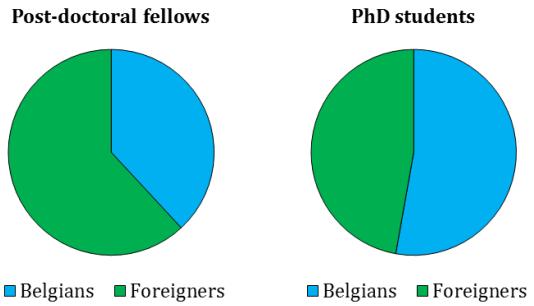
Scientific, technical and administrative staff:

26,1 EFT post-doctoral fellows work for the LDRI and 72 PhD students (45,6 EFT), including 13 "assistants" who have a 50% teaching duty. Part of the other PhD students are cosupervised and thus also attached to other institutes or universities. The 34 technical and administrative people represent ~ 20 EFT technical staff for LDRI.

Staff of the LDRI in 2015

EFT at LDRI	Aca.	Post doct. fellows	PhD students	Techn./ admin staff	Total
Total	12.8	26.1	45.6	20	**104.5**
Non UCL (paid by external resources)	5.6 (FNRS)	23.8	32.9	10.4	**72.7**

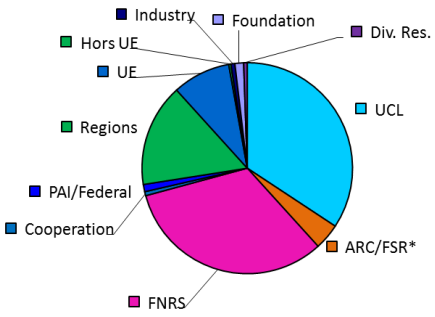
The LDRI staff creates an **international environment:** 47% of the PhD students and 62% of the post-doctoral fellows are foreigners. Moreover, visiting scientists regularly stay at the LDRI for several months.



The background of the scientists is also diversified reflecting the multidisciplinary research. Among the PhD students, most are pharmacists but bioengineers, engineers, chemists, biologists, physicists, MD or masters in biomedical sciences also do their PhD in the LDRI.

The main sources of personnel funding are UCL (34%), the FNRS (32,5%) and regions (16%). The FNRS pays for 6 permanent group leaders, 9 postdoctoral researchers, and 23 PhD students including FRIA and Televie doctoral grants. 13 teaching assistants funded by UCL spend 50% of their time for research.

Sources of funding of personnel attached to LDRI in 2015



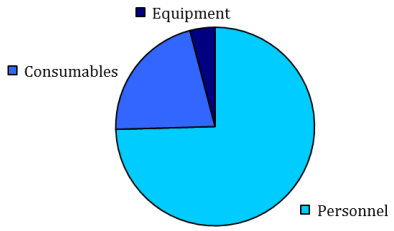
*Competitive grants from UCL,
FSR : Fonds Spéciaux de recherche
ARC : Actions de Recherche Concertées

[1] http://www.uclouvain.be/en-263664.html

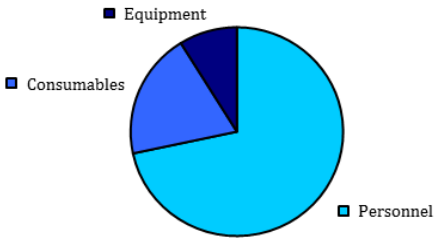
V. Fundings

The annual turnover of the LDRI is approximately 8,15 millions € (8,1 M€ in 2014).

LDRI funding per category of cost in 2015



LDRI funding per category of cost in 2014

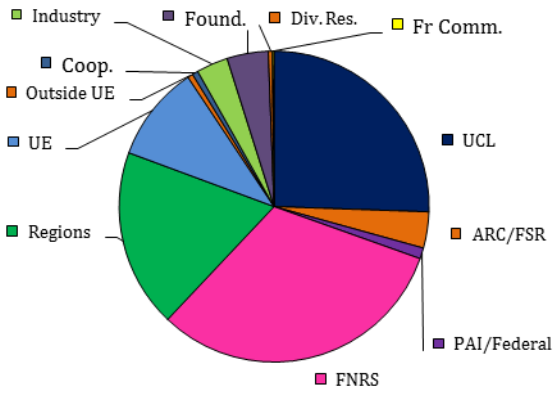


Personnel: EFT at LDRI x mean annual cost per category
Consumables: annual budget for all the projects of the LDRI
Equipment: annual budget for acquisition of new equipment for the whole institute

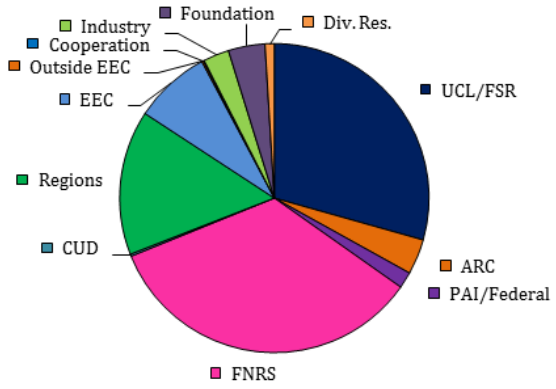
Salaries represent 6,08M€. **Consumables,** 1,74M€. **Equipment,** 0,33M€ in 2015.

The basic funding of LDRI is partly provided by the "ordinary" budget of UCL. The UCL budget mainly covers the salaries: 2,1M€ for the EFT affected to LDRI or 34,1% of the personnel budget. It should be noticed that the staff paid by UCL has also teaching activities not taken into account in the LDRI budget (This is the reason why we considered 0.75 EFT for an academic, 0.5 EFT for an assistant and 0.05 to 1 EFT for technical/administrative staff).

Sources of funding of LDRI (2015)



Sources of funding of LDRI (2014)



The members of LDRI are very active in seeking **financial revenues from third parties**. The external resources increased progressively from 2.49M€ in 2006 to 6,1M€ in 2015. The percentage of the total budget from third parties represents 74,5% of the total budget.

The percentage of funding per category clearly demonstrates that the LDRI is mainly funded by non-profit and research organizations (e.g., FNRS) to support fundamental research and by public funding

(e.g. Walloon region and Brussels region) for applied research. Several European projects are funded. As companies involved in applied research programs can benefit from private-public partnerships e.g. research funded by Walloon region, one to one research contracts (1,5M€ or 18,5% of the total budget in 2015), lead to an underestimation of industrial collaborations in the budget.

VI. Scientific Output

The scientific output of LDRI is mainly estimated from publications in well recognised international journals and training of young researchers. Dissemination of knowledge to the scientific community as well as expertise for Public Health Authorities and /or pharmaceutical, chemical, and biotechnological companies and / or research agencies are not illustrated in an exhaustive way in the present report even though most of the academics are active in these domains.

Publications
(http://www.uclouvain.be/en-263636.html)

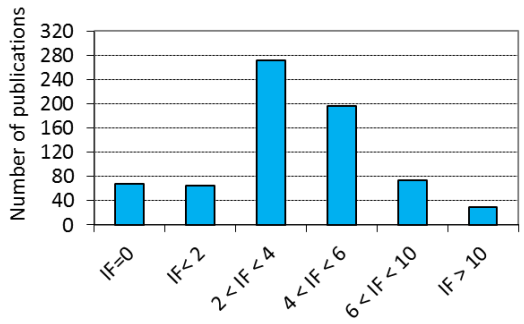
Altogether, all the research groups of the LDRI published 433 scientific articles in international journals or book chapters during the last four years (2012-2015). 8% of these publications (n=35) associate two or more groups belonging to the LDRI.

Moreover researchers have worked to truly break new ground in science and they got publications in general, outstanding journals.

The mean Impact Factor (IF) of the publications during the period 2009-2015 is 4,2 (including review papers and educational papers). The mean IF for original papers is 4,3. 81% of the publications of the LDRI were published in journals with an IF ≥ 2 and 43% in journals with an IF ≥ 4 (between 2009 and 2015).

The distribution of publications per impact factor is shown hereafter.

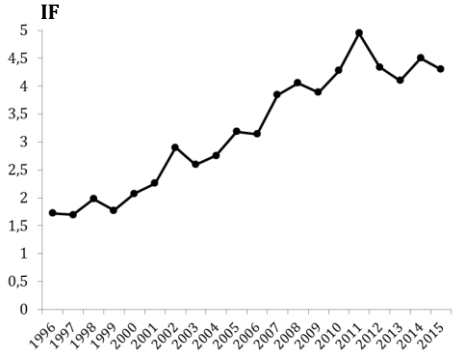
Histograms of the number of publications (period 2009-2015) according to their Impact Factor



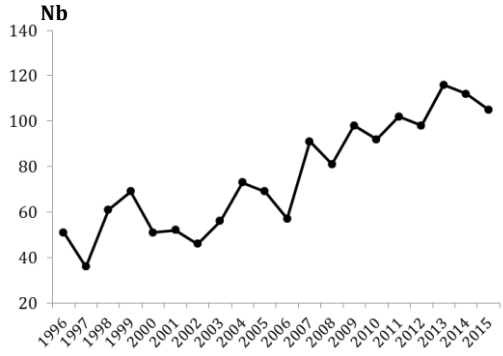
(Papers with IF=0 are book chapters, educational papers or papers published in recent journals without IF)

Over the last 15 years, there was an increase in the number of publications as well as in the quality of these papers, as demonstrated by the global increase in the impact factor of the journals where we publish.

Evolution over the time of the mean impact factor of original papers published by LDRI research



Evolution over the time of the number of annual publications published by LDRI research groups



PhD students' formation

81 PhD students are presently (January 2016) supervised by members of the LDRI. All of them are enrolled in the doctoral school of biomedical and pharmaceutical sciences (orientation: Pharmaceutical Sciences), and 8 PhD theses are in progress under our supervision in other fields (chemistry, engineery, or co-tutorship with foreign universities). 48,5 PhD theses supervised by the LDRI Research Groups were defended during the period 2012-2015.

In addition to doctoral formation, all leaders of research groups are promotors of Master Degree thesis in Biomedical Sciences, Pharmacy, Biology… and of Bachelor's degree dissertations (technicians…).

Seminars-Symposia

(http://www.uclouvain.be/en-318370.html)

The LDRI seminars are targeted at researchers or other professionals having an interest for scientific areas related to drugs in a broad context (from basic sciences to clinical applications). They alternate presentations by junior scientists from the Institute and by senior scientists (from the Institute, from other Institutes within the University, from other Universities in Belgium or abroad, from the Industry).

Expertise

All leaders of research groups are recognised for their expertises by the Authorities of Public Health and/or pharmaceutical, chemical and bio-technological industries, and/or research agencies.

- Conseil Supérieur de la santé, Société de nutrition (Belgium, UK)
- International Life Sciences Institute
- European Safety Food Agency
- European Medecine Agency (EMA)
- Institut Scientifique de santé publique
- Federal Agency for Medecine and Health products
- Federal Agency for Nuclear Control
- Advisory Board related to preclinical or clinical development of new antibiotics
- French National Research Agencies (ANR and AERES)
- Fonds Wetenschappelijk Onderzoek (FWO)

(*The list is illustrative rather than exhaustive*).

Collaborative projects with industries

A lot of collaborative projects with industries are ongoing both with industries (GlaxoSmithkline Biologicals, Astra Zeneca, Danone, Cargill) and SME (Ribx, Trius Therapeutics, Cempra Pharmaceuticals, Thrombogenics, Voco gmbh, Saremco, Heraeus-Kulzer, Coltene, Septodont, Kuraray, Biocodex, Pileje, Normoxys…).

Awards 2014-2015

Laure Bindels: Institut Danone 2015

Patrice Cani: BAILLET-LATOUR Grant for Medical Research 2015

Fabienne Danhier: Prix Paul Van de Velde 2015

Laure Elens: Patsalos prize (best publication 2013-2014, Journal Therapeutic Drug Monitoring) 2015

Amandine Everard: Prix FNRS BIR&D Multi-Disciplinary Thesis Life and Health Science 2015

Raphaël Frederick: Prix d'encouragement en Chimie Thérapeutique (Société Ch. Thér.) – Lab. Servier, 2014

PLENARY LECTURES by EXTERNAL SPEAKERS 2015

Prof. Teun VAN GELDER
Erasmus Medical Center Rotterdam, The Netherlands
Therapeutic drug monitoring of Myco-phenolic acid in renal transplantation and potential relevance of pharmacogenetics

Dr Sona KUCHARIKOVA
Department of Molecular Microbiology, VIB, and Laboratory of Molecular Cell Biology, Institute of Botany and Microbiology, KULeuven, Leuven
Candida albicans – Staphylococcus aureus interaction during in vitro and in vivo dual species biofilm development

Dr Piet HERDEWIJN
Medicinale Chemie, Rega Institute, KULeuven, Leuven
Towards synthetic genes and organisms

Lisa BURRY
Clinical Pharmacy Specialist, Dept of Pharmacy, Mount Sinaï Hospital, Toronto, Ontario
Sedation and delirium in critically ill patients: a program of research

Dr Marc-Emmanuel DUMAS
Imperial College, London
Harnessing the Metabolome with the Genome, the Metagenome and the Interactome for Personalised Healthcare

Joost HAELEWYN
Application specialist Biacore
Surface Plasmon Resonance: label-free screening and detailed kinetic & affinity characterization of (bio-)molecular interactions

Dr Christian KLEUSCH
NanoTemper Technologies
Some like it hot – Biomolecule Analytics using Microscale Thermophoresis (MST)

Prof. Will PALIN
Oxford and Wessex Deaneries, Oxford, UK
The potential for novel photo-therapeutic treatments in dentistry

Prof. Xavier DE BOLLE
Microorganisms biology research unit, University of Namur, Namur
Brucella abortus coordinates cell cycle and infection

Prof. Jean-Marie MALOTEAUX
Président du comité d'éthique hospitalo-facultaire des cliniques universitaires Saint-Luc
Ethique en essais cliniques : situation actuelle et règlement européen futur en 2016

Prof. Claude KNAUF
Université Paul Sabatier, Institut des Maladies Métaboliques et Cardiovasculaires (I2MC), European Associated Laboratory, Neuro-Microbiota (INSERM/UCL)
Targeting the duodenal contractility to treat type 2 diabetes?

SECTION II – RESEARCH GROUP PRESENTATION

I. Advanced Drug Delivery and Biomaterials (ADDB)
II. Bioanalysis and Pharmacology of Bioactive Lipids (BPBL)
III. Medicinal Chemistry (CMFA)
IV. Pharmacognosy (GNOS)
V. Metabolism and Nutrition (MNUT)
VI. Biomedical Magnetic Resonance (REMA)
VII. Translational Research from Experimental and Clinical Pharmacology to Treatment Optimization (TFAR)

Advanced Drug Delivery and Biomaterials (ADDB)

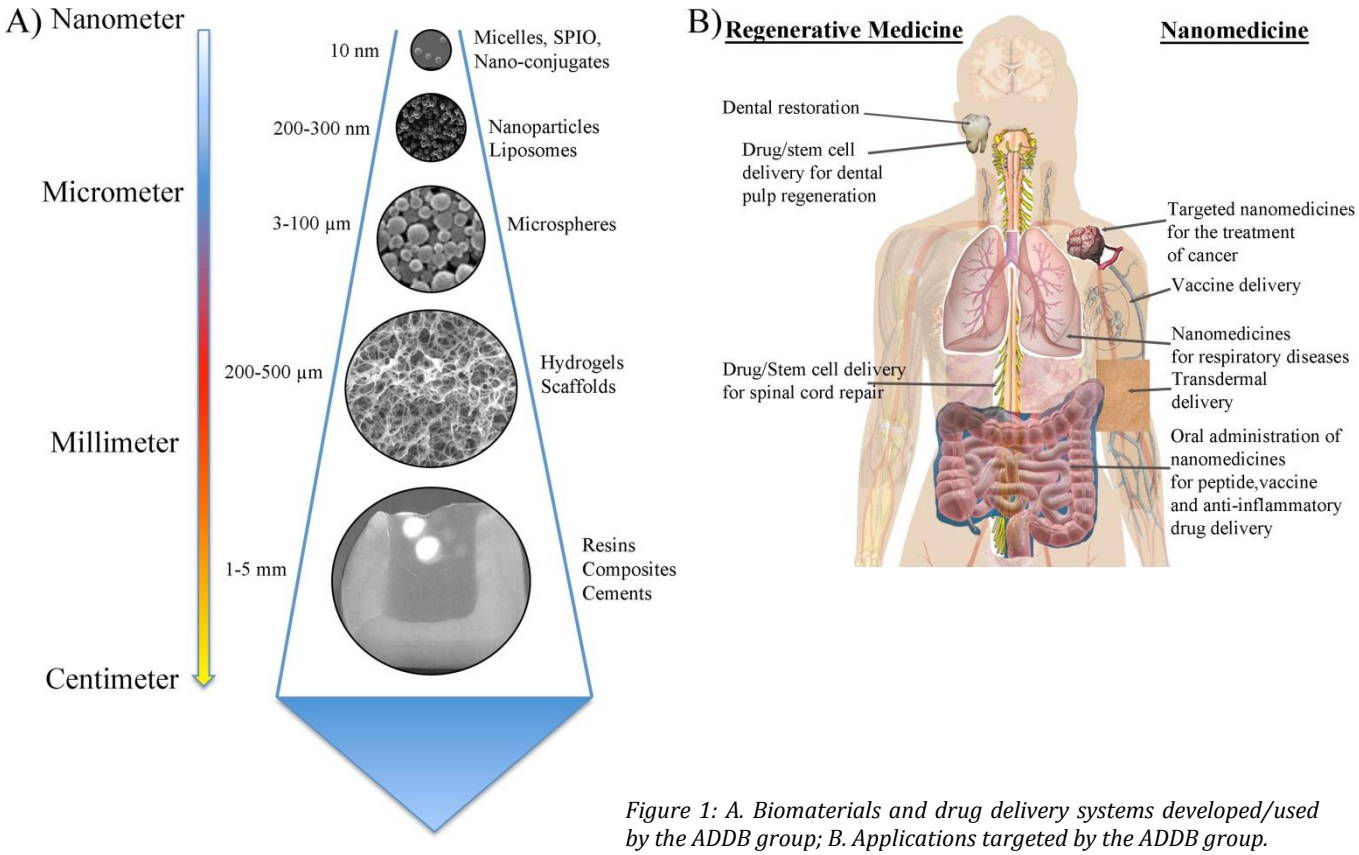


Figure 1: A. Biomaterials and drug delivery systems developed/used by the ADDB group; B. Applications targeted by the ADDB group.

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Advanced Drug Delivery and Biomaterials (ADDB)

Post-Doctoral fellows
Beloqui A.
Danhier F.
Freches D.
Leprince J.
Loira Pastoriza C.
Patil H.P.
Vanacker J.
Vandermeulen G.
Bianco J.

PhD Students
Bastiancich C.
Beauquis J.
Bouttefeux O.
Cherredy K.
Carradori D.
De Berdt P.
Ganipineni P.
Germain L.
Gilli M.
Guichard M.-J.
Hardy C.
Hollaert T.
Jacobs D.
Jully V.
Kandalam S.
Lambricht L.
Lasserre J.
Lopes A.
Luo T.
Mengnan Z.
Randolph L.
Setbon H.
Tatic N.
Twala L.
Viswanath A.

Adm. & Techn. Staff
Callier M.
Dos Santos A.
Lecouturier N.
Machado M.
Staub A.
Ucakar B.
Vandiest J.-P.
Vanverenberg K.

Website ADDB: http://www.uclouvain.be/en-269736.html

Address: Bâtiment Van Helmont – Tour 73, 2ème étage, avenue E. Mounier 73, B1.73.12, B-1200 Brussels.
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The objective of our research is to use drug delivery systems and biomaterials as a mean to improve therapeutic outcomes of drugs. We develop drug delivery systems going from nano-scale, through micro-scale up to macro-scale (Figure 1A).

Our different research applications can be gathered into two research themes (Figure 1B):

1. Nanomedicines:

a. Cancer: this theme focuses on targeted theranostic nanoparticles loaded with anticancer drugs or siRNA, adjuvanted recombinant antigens and gene vaccines.

b. Mucosal delivery routes: this theme includes the research on oral delivery using nanomedicines, cutaneous delivery and pulmonary delivery.

2. Regenerative medicine: *this theme focuses on tissue regeneration and restoration and gathers the research on spinal cord regeneration, dental restoration and skin wound healing.*

NANOMEDICINES FOR TARGETED DRUG DELIVERY (V. PRÉAT, F. DANHIER, A. BELOQUI)

Polymeric and lipidic nanomedicines are developed for the administration of poorly water soluble drugs, peptides, vaccines and nucleic acids. Our research mainly focuses on (i) oral delivery of lipidic and polymeric nanoparticles loaded with drugs, proteins or antigens (ii) intravenous delivery of drug-loaded of nanoparticles targeting the tumoral endothelium and cancer cells.

Different strategies of nanoencapsulation for the oral delivery of drugs have been compared with a special emphasis on their mechanisms of drug transport (*Figure 2*). Therefore, we have developed *in vitro* models of intestinal epithelium and follicle-associated epithelium containing M cells. The transport of PLGA-based and chitosan-based nanoparticles is endocytosis-mediated and enhanced by M cells, in particular if RGD ligand that targets integrin overexpressed at the apical pole of M cells are grafted. In contrast, the transport of self-assembling lipid-based systems and nanostructured lipid carriers is not enhanced by M cells. Several biomedical applications of oral delivery of nanomedicines are investigated: i) enhancement of poorly soluble drug delivery e.g. antimicrobial and antimalarial drugs ii) mucosal immunization with untargeted nanoparticles and nanoparticles grafted with RGD or mannose iii) treatment of experimental colitis iv) oral peptide delivery.

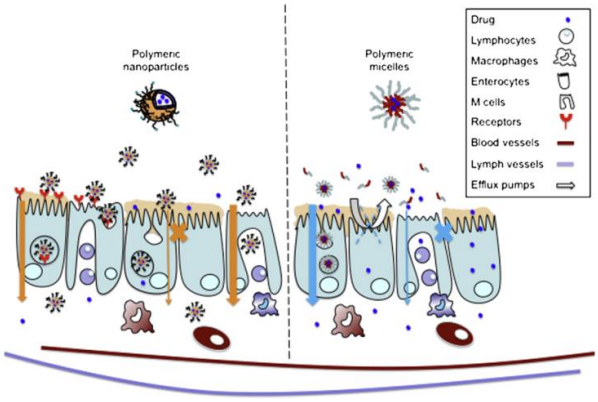

Figure 2: Schematic representation of the fate of polymeric nanoparticles and micelles after oral delivery

Several main mechanisms of delivery of drug-loaded nanoparticles to tumors have been reported (*Figure 3*): (i) passive targeting through leaky vasculature surrounding the tumors, described as the enhanced permeability and retention effect (EPR) (ii) "active" targeting by grafting specific ligands of cancer cells or angiogenic endothelial cells to the surface of the

nanocarrier (iii) magnetic targeting of SPIO (small paramagnetic iron oxides) loaded nanoparticles. We formulated various nanocarriers (micelles and untargeted or targeted nanoparticles) loaded with several anti-cancer drugs to specifically target tumors and improve the therapeutic index of anti-cancer drugs by nanomedicines. For example, PLGA-based nanoparticles formulated for the delivery of paclitaxel, a new cyclin dependent kinase inhibitor and doxorubicin induced a higher regrowth delay of tumors *in vivo* than free drugs. Micelles allowed a better therapeutic response *in vivo* than nanoparticles, due to their more adapted size for the EPR effect (20 nm) than nanoparticles (200 nm). Exploiting the $\alpha_v\beta_3$ integrin overexpression by tumoral endothelium and tumor cells, we designed PLGA-based nanoparticles grafted with the RGD peptide and demonstrated the "active" targeting of these PLGA-based nanoparticles. We formulated multi-functional nanoparticles for the encapsulation of a therapeutic drug and a contrast agent (SPIO) that can be targeted by magnets and significantly enhanced drug biodistribution and tumors. Our current projects are focussed on the mechanisms of action of nanomedicines, in particular their effect on the tumor microenvironment. Anticancer drug-loaded nanomedicines are developed for the treatment of glioblastoma. Coentrapment of melanoma-associated antigens and Toll like receptor ligands in mannose-functionalizes nanoparticles potentiated Th1 immune response and decreased tumor growth in therapeutic settings.

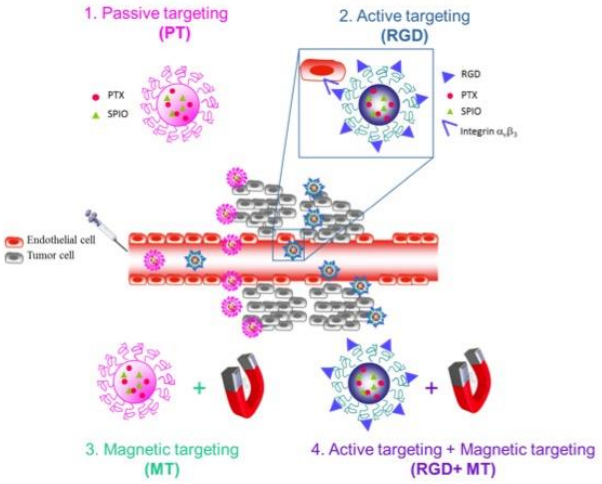

Figure 3: Passive, active and magnetic targeting of anticancer drug-loaded nanomedicines

NANOMEDICINES FOR PULMONARY DELIVERY (R. VANBEVER)

The research aims at improving the treatment or prophylaxis of severe respiratory diseases by designing nanomedicines to enhance the local efficacy of drugs. Our approaches include i) the preparation of polyethylene glycol (PEG)-drug conjugates to sustain drug release within the lung, and ii) the formulation of liposomes to target vaccines to lung dendritic cells.

Inhalation aerosols offer a targeted therapy for respiratory diseases. However, the therapeutic efficacy of inhaled drugs is limited by their rapid clearance in the lung (*Figure 4*). We synthesized PEG-paclitaxel ester conjugates with the aim to achieve sustained release of paclitaxel in the lung. These conjugates were characterized physicochemically and showed good stability in phosphate buffer and in bronchoalveolar lavage, but hydrolyzed quickly in mouse serum. The conjugates showed cytotoxicity to B16-F10 melanoma cells and Lewis lung carcinoma cells but less than Taxol, which is the commercial

paclitaxel solution. The conjugates will be further investigated in vivo on B16-F10 lung metastasis mouse model to test the sustained drug release as well as the anti-tumor efficacy.

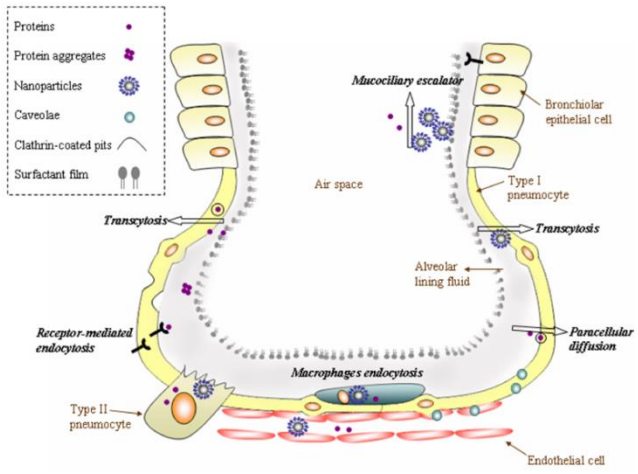

Figure 4: Schematic view of the fate of drugs in the lungs (from Todoroff & Vanbever, Curr. Opin. Coll. Interf. Sc., 2011)

Anti-IL17 F(ab')$_2$ and anti-IL13 Fab' antibody fragments were conjugated to a large PEG chain and conjugation was shown to greatly prolonged the presence of these fragments within the lungs of mice. The prolonged pulmonary residency of the anti-IL-17 PEG-F(ab')$_2$ translated into an improved efficacy in reducing lung inflammation in a murine model of house dust mite-induced lung inflammation. PEGylated proteins were principally retained within the lung lumen rather than the nasal cavities or lung parenchyma. PEG increased pulmonary retention of antibody fragments through mucoadhesion and escape from alveolar macrophages rather than increased hydrodynamic size or improved enzymatic stability.

We have also applied this PEGylation strategy to recombinant human deoxyribonuclease I (rhDNase). rhDNase is the mucolytic agent most widely used for the treatment of respiratory disease in cystic fibrosis. However, rhDNase I is rapidly cleared from the lungs, which limits its therapeutic efficacy and implies frequent dosing. rhDNase was monoPEGylated on the N terminal residue and the conjugated enzyme preserved the full enzymatic activity of the native protein. PEGylated rhDNase was retained in the lungs for more than 10 days, compared to a few hours for unconjugated rhDNase.

We develop liposomes for targeting vaccines to lung dendritic cells. Nanoliposomes were prepared with cationic lipids presenting immunostimulatory capacities. These formulations were shown to successfully co-encapsulate both antigenic peptides and adjuvants with high loading efficiency. Their sizes were comprised between 150 and 180 nm and a sustained release of antigens from the nanoliposomes over several hours was obtained. The fate of liposomes-encapsulated peptides in the respiratory tract is currently studied and the protection these formulations afford is currently assessed in vivo in murine models.

DRUG AND CELL DELIVERY IN TISSUE ENGINEERING (A. DES RIEUX)

The research aims at developing implants (hydrogels, polymeric scaffolds) delivering growth factors, drugs and cells that provide sustained delivery of bioactive molecules, support survival, infiltration and proliferation of cells for tissue engineering, and in particular spinal cord injury (*Figure 5*).

Our group has gained expertise in drug delivery to the spinal cord that we would like to combine with transplantation of adult mesenchymal stem cells; more particularly human dental stem cells. Indeed, human dental stem cells display superior neural stem cell properties than bone marrow-

derived mesenchymal stem cells since they originate from the neural crest. We have evaluated the impact of hypoxia, an important pathological cue in the injured spinal cord, on differentiation of dental cells derived from the human apical papilla (SCAPs). In our most recent works, we have incorporated SCAPs in different hydrogels that are now ready to be tested for their potential in spinal cord repair strategies.

1. Sustained delivery of VEGF and GDNF from injectable hydrogel

We evaluated the effect of VEGF and GDNF delivery, free or encapsulated, from an alginate: fibrinogen hydrogel injected in a rat spinal cord hemisection model.

Local VEGF delivery from alginate: fibrinogen hydrogel gelifying *in situ* induced angiogenesis and neurite growth but no functional improvement. The animals treated with free GDNF-loaded hydrogel experienced superior functional recovery compared to the animals treated with GDNF microsphere-loaded hydrogels and non-treated animals *(in collaboration with Prof. Blanco-Prieto, Navarra University, Spain, Drs Schakman and Deumens, UCL, IoNS).*

2. Influence of hypoxia on the differentiation potential of SCAPs

When stem cells are implanted *in vivo* as part of strategies for regenerative strategies, it is not unusal for the cells to spend at least a week in hypoxia before a new vasculature develops. Our objective was to assess the impact of hypoxia on SCAP proliferation, differentiation and growth factor expression/production. We worked with a well-characterized primary cell line (RP89 cells) provided by our collaborator, Pr. Anibal Diogenes, from University of Texas at San Antonio (USA). We have grown SCAPs in hypoxia ($1\%O_2$) and in normoxia and compared growth rate and expression of stemness genes (SURVIVIN, CD90, and CD105), osteogenic (RUNX2 and ALP), adipogenic (ALBP), neurogenic (CNP, NSE, and SNAIL) lineages genes and growth factor genes (VEGFA, VEGFB, BFGF, TGF-b1, GDNF, and NT3). Impact of hypoxia on neuro-, osteo- and adipo-differentiation of SCAPs was also determined. We showed a clear impact of hypoxic culture conditions on the differentiation potential of SCAPs. In particular, the up-regulation of neuro- and osteospecific genes and the pro-angiogenic factor in SCAPs cultured in basal medium supports the potential of SCAPs to promote tissue regeneration. Hypoxia was also particularly favorable for neurodifferentiation, which is promising for neuroregenerative events. We are now looking in more details at the potential of hypoxia cultured SCAP for spinal cord repair.

3. Survival, proliferation and differentiation of SCAPs in different hydrogel

Cell-based therapies are the most common approaches in regenerative medicine. One important characteristic for the ideal scaffold to be used in regenerative procedures is to provide appropriate conditions to ensure cell attachment, growth and differentiation. Despite significant advances in the use of dental stem cells in regenerative procedures, no study has previously evaluated the effect of SCAP encapsulation in alginate, Corgel®, and fibrin hydrogels on cellular survival, proliferation and neurodifferentiation. We studied the impact of SCAP encapsulation in different hydrogels. Incorporation in fibrin hydrogels of medium and high fibrinogen concentrations maintained SCAP viability, supported SCAP proliferation and neuro-differentiation *in vitro* but also allowed SCAP proliferation, collagen secretion and angiogenesis *in vivo*. Depending on the objective (SCAP viability, proliferation,

growth factors secretion or neuro-differentiation) but also taking into account specific constrains (residence time, injectability, gelification time), a different fibrin formulation can be selected among the tested combinations. Fibrin hydrogels prepared with 30 mg/ml or 50 mg/ml of fibrinogen could be efficient and suitable SCAP scaffolds for CNS regeneration. Regarding SCAP incorporation in different alginates and Corgel®, our results demonstrated the important role of hydrogel properties on SCAP viability. This study highlights that not a single property, but the appropriate combination of surface (presence of adhesion sites and hydro-phobicity) and mechanical characteristics dictate SCAP fate.

Another promising type of hydrogel is extra cellular matrix (ECM)-based hydrogels. This type of hydrogel has been developed by Pr. Badylak (University of Pittsburgh, USA) and Pr. Shakesheff (University of Nottingham, UK) with whom we collaborate. ECM-based hydrogels present the advantages of being thermosensitive, thus injectable, and to share the same origin (tissue-wise) than the tissue to repair. ECM hydrogels have been obtained from various tissues like bone, bladder, brain and spinal cord. In the scope of an Erasmus Mundus program (NanoFar), a joint PhD thesis between the University of Nottingham and the Université catholique de Louvain is currently ongoing and deals with the potential of porcine spinal cord ECM hydrogel for spinal cord repair. Up to know we have studied the impact of SCAP culture in bone ECM and spinal cord hydrogel *in vitro*.

4. Influence of SCAP on spinal cord repair

In order to evaluate the impact of SCAP on spinal cord repair, we have delivered SCAP in fibrin hydrogel or a whole apical papilla in a spinal cord hemisection model and observed a significant functional recovery of the rats after 6 weeks when they were treated with apical papilla.

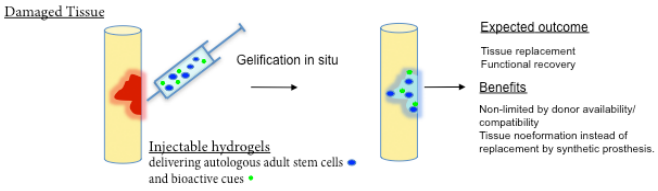

Figure 5: Drug and cell delivery in tissue engineering

NUCLEIC ACID DELIVERY (V. PRÉAT, G. VANDERMEULEN)

We aim to develop formulation (nanoparticles) and physical methods (electroporation) for the delivery of DNA and RNA (siRNA, mRNA) with a particular interest in vaccination and cancer treatments (*Figure 6*).

Electroporation of DNA was optimized to deliver plasmid vaccines into the skin or the muscle. This potent delivery method allows high level of expression. Optimised plasmids encoding tumor antigens elicited humoral and cellular immune response and induced tumor control or regression. Electroporation of an antiangiogenic plasmid encoding the recombinant domain of ADAM-15 reduced tumor growth and modified the tumor microenvironment, leading to synergy with radiotherapy. Electroporation of plasmid coding for host defense peptides promoted wound healing in healthy and diabetic mice models.

Nanoparticles loaded with DNA and siRNA have been developed and showed good transfection efficiency *in vitro*. We are currently developing targeted nanoparticles for a specific delivery of siRNA to tumors *in vivo*.

In a near future, our physical and formulation approaches will be extended to deliver DNA, siRNA, mRNA with a special emphasis on cancer treatment.

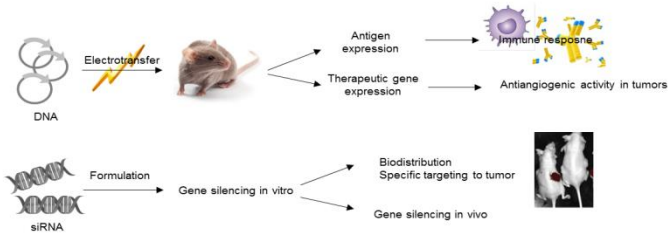

Figure 6: Plasmid DNA and siRNA delivery

DENTAL REGENERATIVE and INNOVATIVE MATERIALS (G. LELOUP)

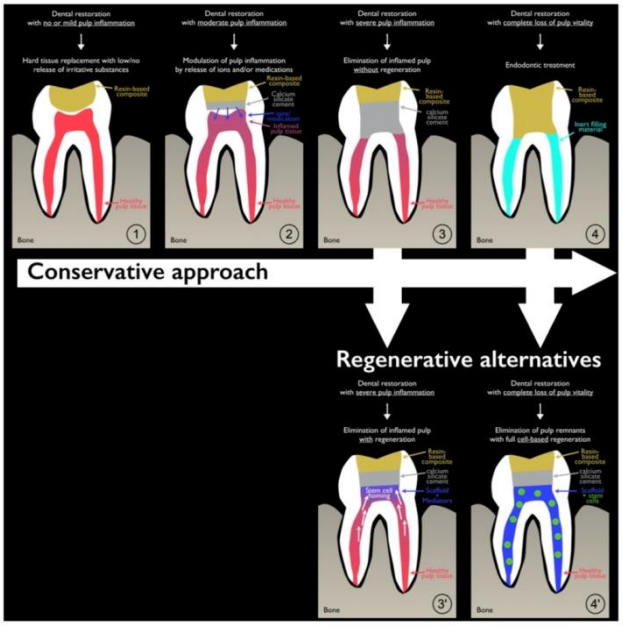

Figure 7: Nowadays behaviours for the treatment of tooth decay.
1) In case of tooth decay and a healthy pulp, a resin-based composite is used. 2) In case of tooth decay with an inflamed pulp, a calcium-silicate cement is applied on the pulp before the resin-based composite. 3) When the pulp is partially necrotic, this part is removed and replaced by a combination of calcium-silicate cement and resin-based composite. 4) When the complete pulp is necrotic, it is completely removed and replaced by inert filling material. Future behaviours for the treatment of tooth decay. 3') When the pulp will be partially removed, it will be replaced by a hydrogel loaded with growth factors in order to attract stem cells from the remaining pulp. 4') When the pulp will be completely removed, it will be replaced by a hydrogel loaded with stem cells in order to re-create a new dental pulp tissue.

In the treatment of tooth decay, restorative dental materials are required to exhibit excellent mechanical, biological properties and most uniquely, display good aesthetics. The research carried out focuses 1) on the characterization of currently available commercial materials, in relation with clinical requirements and 2) on developing new biomaterials for tooth restoration, from a conventional conservative but also from a more advanced regenerative standpoint.

STRATEGIES AND RESULTS

1) Conservative approach

The use of restorative materials allows for relatively fast treatments as they may be implemented directly in the oral cavity in a matter of minutes. They are also highly versatile. However several concerns exist with regards to the suitability of some materials in terms of mechanical or biological properties. Additionally the very mechanisms responsible for the setting of materials or interactions with the biological are little understood.

a) <u>In vitro-methods</u>

We are continuously invested in determining the most suited set of characterization methods to properly analyze both mechanical and biological properties of commercial materials, leading to innovative experimental research. Our most recent results describe the setting kinetics and mechanical properties of ultra-fast polymerizing resin composites, based on a monoacylphosphate photoinitiator and bioactive calcium silicate cements (Figure 6, item 2 or 3). In collaboration with Pr. Möginger (University of Bonn-Rhein, Germany), an innovative combination of

characterization techniques was set up, allowing for a precise analysis of polymerization kinetics in heavily filled composites.

b) In vitro-material development

The formulation of resin composites is fine-tuned ([photoinitiator], resin composition, etc) to quicken kinetic, increase longevity and bring mechanical properties close to that of hard tissues. The use of micro hydroxyapatite particles and amorphous CaP nano particles is currently being investigated for the release of Ca^{2+} and PO_4^{2-} with antibacterial and re-mineralizing potential. The impact of their introduction in model formulations on kinetics and mechanical properties is investigated. Additionally, ceramics are investigated for their use as alternatives of resin composites following root canal treatment (Figure 6, item 4).

c) In vitro-material/cell interactions

The interactions with pulp tissues and oral commensal bacteria are also researched. The potential of apatite-loaded resin composites is being evaluated, aiming both at *S.mutans/S.gordonii* biofilm reduction and increased dental pulp stem cells (DPSC) viability (induced osteo-differentiation is also analyzed). Further, as resin composites do not polymerize completely, the toxicity of monomers and un-reacted compounds on DPSC is investigated. Even in the absence of toxicity, some monomers may still induce oxidative stress and genotoxic effects. Methods are being developed to quantify ROS production and osteo-differentiation inhibition on a large number of samples.

d) Clinical work

As a result of strong collaborations with the dental clinics, several studies are currently under way, focusing on the analysis of the suitability of resin composites for the treatment of large cavities, in a retrospective manner. Another study underway was designed to investigate the suitability of calcium silicate cement for pulp capping (Figure 6, item 3).

2) Regenerative approach

In modern dentistry, there is currently a paradigm shift from restorative procedures to strategies based on regenerative medicine. In this context, alternatives to current clinical restorative strategies where pulp tissue is partially or completely lost (irreversibly inflamed and necrotic dental pulps) must we designed by combining bioactive matrices and dental stem cells in a clinically relevant way.

a) Cells

Dental stem cells are mesenchymal stem cells that may be collected in large amounts from dental tissues. Such cells display a higher proliferation rate than bone marrow stem cells and have better neural and epithelial properties as they originate from the neural crests. Additionally dental stem cells can differentiate in multiple cells types, like osteo- odonto-, adipo-, neuro-, chondroblast-like cells… Among dental stem cells, we selected dental pulp stem cells (DPSCs) and stem cells from the apical papilla (SCAP) for their potential. While we have worked with SCAP (RP89 cell line), originating from one patient and obtained from Dr. Diogenes (University of Texas, USA), we recently created a pool of DPSC and SCAP from 10 different patients. These cell pools will be fully characterized by cell-surface markers analysis, by differentiation potential and by stem cell gene expression and used as an internal standard for all of our work. Such efforts will allow us to have a much genetically diverse and relevant cell source.

b) Scaffold

For the regenerative approach, cells must be properly delivered. The design of an "ideal" bioactive matrix is thus necessary. This one would be biocompatible, injectable and would ideally resemble the native pulp tissues in terms of mechanical properties and allow cell invasion, survival and proliferation. Therefore, we will test *in vitro* different hydrogels, which will be provided through different collaborations (Prof. Anne des Rieux, UCL; Prof. Berit Strand, NTNU, Norway; Prof. Patrick Henriet, UCL; Prof. Christine Dupont, UCL).

Fibrine/Alginate hydrogels are currently being investigated, testing for DPSC attachment and viability on the medium-term. Once an « ideal » bioactive matrix is designed, it will be implemented in two different regenerative strategies and tested *in vitro*/*in vivo*:

-Dental pulp stem cell homing from residual dental pulp tissue in case of *partial* pulp tissue removal, through the injection of a bioactive scaffold loaded with factors like SDF1, bFGF and TGF-B (Figure 6, item 3'),

-Exogenous dental pulp stem cell delivery in case of *complete* pulp tissue loss, to regenerate the lost tissue volume into a vascularized, innervated and functional de-novo dentin-pulp complex (Figure 6, item 4').

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AWARDS 2014-2015

Préat Véronique
Highly cited researcher 2015

Patil Harshad Padmanabh
Best poster presentation award

THESIS
2015

Damien Jacobs "Characterization of Wallerian Degeneration bu In Vivo Diffusion Compartment". Director: B. Macq; Co-directors: A. des Rieux & B. Gallez

Chereddy Kiran Kumar "Drug-loaded PLGA based nanoparticles for wound healing" Director: Véronique Préat; Co-Director: Gaëlle Vandermeulen

Schleich Nathalie "Targeting strategies of theranostics" Director: Véronique Préat; Co-Director: Bernard Gallez

THESIS
In progress

Bastiancich Chiara "Nanoparticles-loaded hydrogel for the treatement of Gliobastoma" Director: Véronique Préat; Co-directors: Frédéric Lagarce, Fabienne Danhier

Beauquis Julien "Understanding and management of the mechanisms of pulp inflammation" Co-directors: Gaëtane Leloup, Julian Leprince

Carradori Dario "New peptide-nanovector delivery system in neural or mesenchymal stem cells" Director: Pr Joël Eyers (Université d'Angers); Co-Director: Anne des Rieux

De Berdt Pauline "Influence des cellules souches dentaires sur la régénération de la moëlle épinière" Director: Anne des Rieux; Co-Director: R. Deumens

Germain Loic "Effect of biocompatible surfaces presenting mechanical nano-heterogeneities on Adipose-Derived Mes-

enchymal Stem Cells neurodifferentiation" Director: Anne des Rieux; Co-Director: Christine Dupont

Ganipineni Pallavi "Comparaison de différentes stratégies de ciblage tumoral: évaluation in vivo de nanoparticules à visée theranostiques " Director: Véronique Préat; Co-Director: Fabienne Danhier

Gilli Mathieu "Paving the way for tomorrow's dentistry with hydrogel-based strategies for pulp tissue regeneration" Director: Gaëtane Leloup; Co-Director: Julian Leprince & Julie Vanacker

Guichard Marie-Julie "Développement d'un agent mucolytique innovant pour la mucoviscidose " Director: Rita Vanbever ; Co-Director: Teresinha Leal

Hardy Chloé "Optimisation of the bonded indirect dental restorations" Co-directors: Gaëtane Leloup, Julian Leprince

Hollaert Thibaut "Optimization of dentin-substitute materials" Co-directors: Gaëtane Leloup, Julian Leprince

Jully Vanessa "Development of a High-Throughput Screening Platform for the Formulation of Alum Adjuvanted Vaccines" Director: Véronique Préat; Co-Director: Frédéric Mathot (GSK Biologicals)

Kandalam Saikrishna "Nano and micro tailoring of biomimetic and pharmacologically active biomaterials (PAMs) combined to adult stem cells for spinal cord injury" Director: Pr Montero-Menei (Université d'Angers); Co-Director: Anne des Rieux

Lambricht Laure "Nanoparticulate drug delivery systems for cancer vaccines" Director: Véronique Préat; Co-Director: Gaëlle Vandermeulen

Lasserre Jérôme "Evaluation of new strategies to control dental biofilms and related diseases" Director: Michel Brecx; Co-Director: Gaëtane Leloup

Lopes Alessandra "Towards new strategies to improve DNA cancer vaccine performance" Director: Véronique Préat; Co-Director: Gaëlle Vandermeulen

Luo Tian "Pulmonary delivery of sustained-release forms of paclitaxel for improved therapy of lung cancer" Director: R. Vanbever; Co-director: C. Bosquillon (U Nottingham)

Mengnan Zhao "Anticancer drug loaded hydrogel for the treatment of glioblastoma" Director: V. Préat; Co-director: F. Danhier

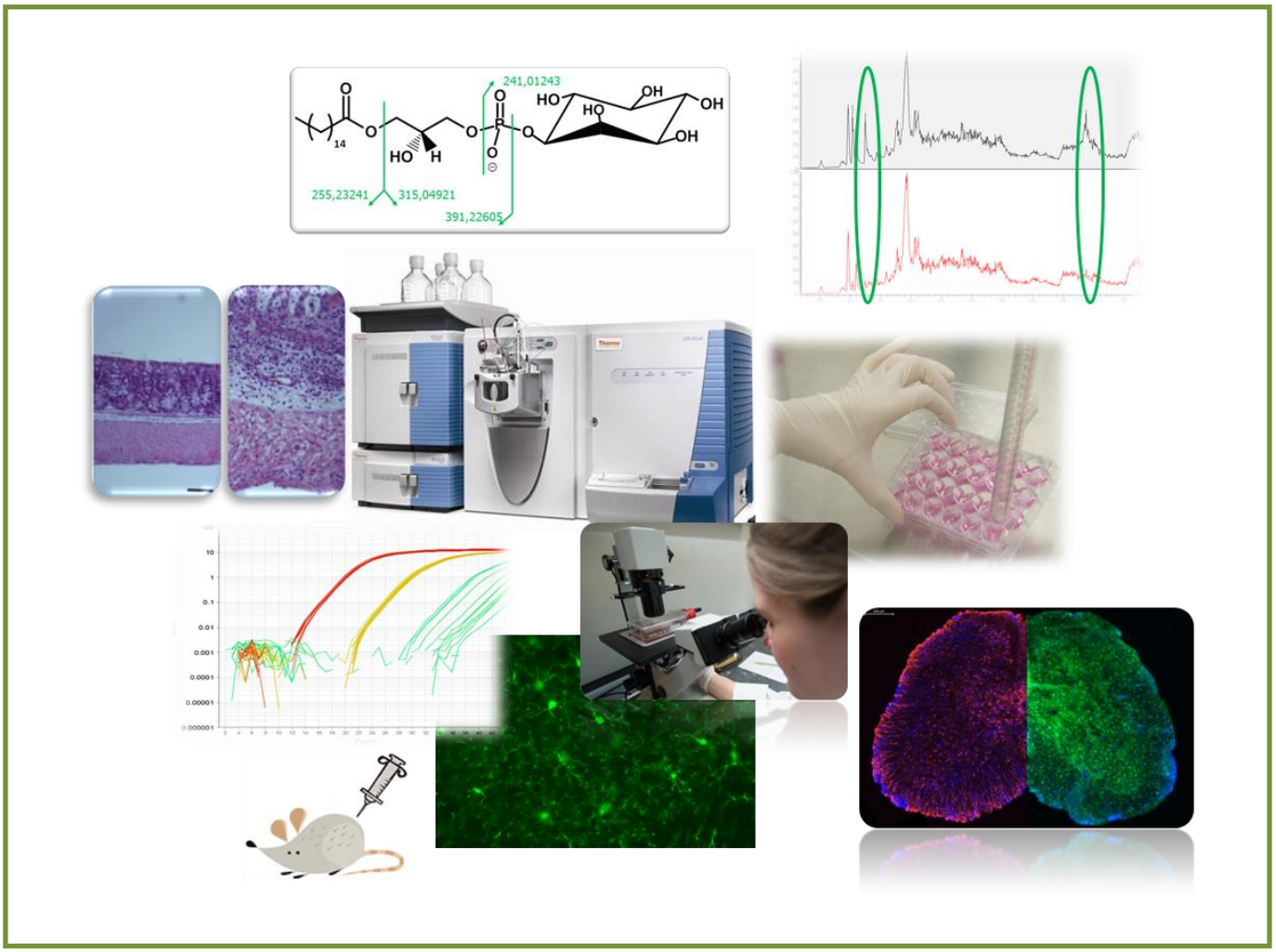
Randolph Luc "Development of a new dental composite with optimized antibacterial and mechanical properties" Co-directors: Gaëtane Leloup, Julian Leprince

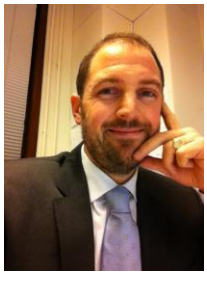
Setbon Hugo "Matériaux bioactifs en endodontie : aspects fondamentaux et applications cliniques" Co-directors: Gaëtane Leloup, Julian Leprince

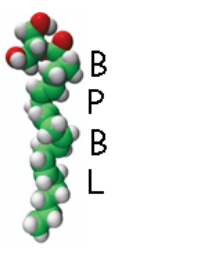
Twala Lungile "Polysaccharide-based nanoparticles for oral delivery of peptides" Director: Maria José Alonso (U Santiago di Compostela); Co-Director: V. Préat

Viswanath Aishwarya "Dental stem cell delivery through new injectable matrices for spinal cord regeneration" Director: Anne des Rieux; Co-Director: Kevin Shakesheff (U Nottingham)

Bioanalysis and Pharmacology of Bioactive Lipids (BPBL)







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Our group is interested in understanding the roles of bioactive lipids both in physiological and pathological situations mainly related to inflammation.

*Indeed, lipids are essential constituents of biological membranes and control numerous cellular activities. An increasing number of lipids are shown to possess biological activities, thus behaving as transmitters or mediators. A large proportion of these "**bioactive lipids**" act by binding and activating their own receptors, and have their levels tightly regulated by specific enzymes. A prime example of such bioactive lipid signaling system is represented by the endocannabinoid system, one of our major research interests.*

*Thus we investigate the role of bioactive lipids (1) by setting up analytical methods (e.g. HPLC-MS) allowing the quantification of their endogenous levels and (2) by interrogating the role of selected bioactive lipids in cellular and in-vivo models of inflammation-related diseases (Figure 1). **The overall aim of the group is to identify novel lipid-related therapeutic targets**.*

RESEARCH RESULTS

1. Quantification of bioactive lipids.

The biological activities of most bioactive lipids are controlled by the balance between their production and degradation. However, because numerous bioactive lipids are produced and degraded by multiple pathways, measuring the expression or activities of the enzymes involved is often not enough. Thus it is essential to directly quantify their endogenous levels, as these represent the final result of the synthetic and degradation pathways. We therefore develop analytical methods that will help us understand the role and implication of these bioactive lipids in pathophysiology.

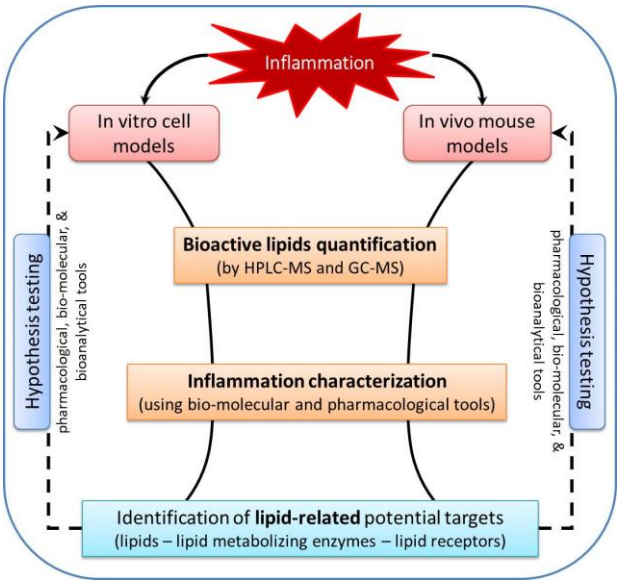

Figure 1: The overall strategy of our group centers on the identification of lipids and lipid-related potential targets in inflammatory settings. Bioactive lipids are selected either on the basis of reported effects or following their identification in lipidomics studies in the lab. The effects of these bioactive lipids are assessed, in vitro or in vivo, to determine their potential impact on inflammation. Once interesting lipids are selected and their effects identified, we turn to the identification of potential means to control their effects in vivo, for example by using agonists or antagonists of their receptors, or interfering with their biosynthetic and degradation pathways using inhibitors.

1.1. Validation of an HPLC-MS method for quantifying oxysterols, ceramides and endocannabinoids.

These three families of bioactive lipids are suggested to be involved in obesity and metabolic syndrome. To facilitate the quantification of these potentially interconnected lipids, we have developed and validated a HPLC-MS method allowing for their simultaneous quantification from tissues (Mutemberezi et al. Anal Bioanal Chem, 2016). A critical step of the method is the

extraction and purification of as many oxysterols as possible. Indeed, oxysterol quantification methods are affected by analytical artifacts due to the oxidation of cholesterol during the analytical procedure. The sensitivity and specificity of the method allow us to quantify the 23 analytes of interest in a number of different tissues (including the liver, adipose tissue and plasma). We found that 16 weeks of high-fat diet strongly impacted the hepatic levels of several oxysterols, ceramides, and endocannabinoids and that a partial least-squares discriminant analysis (PLS-DA) based on the variations of the hepatic levels of these 23 bioactive lipids allowed for differentiating the lean mice from the obese mice (Figure 2).

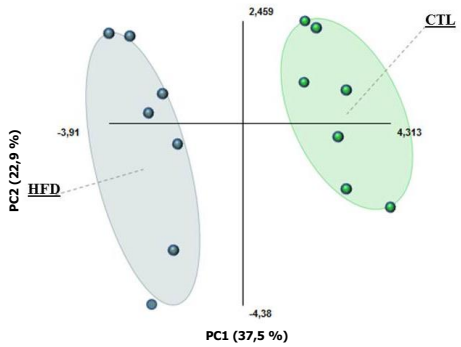

Figure 2: Partial least-squares (PLS) discriminant analysis based on the hepatic levels of oxysterols, ceramides and endocannabinoids found in control and obese mice. Each dot on the graph represents a mouse. (adapted from Mutemberezi et al. Anal Bioanal Chem, 2016)

1.2. Quantification of bile acids from mouse and human plasma.

Next to the oxysterols, bile acids are the second family of important bioactive lipids issued from cholesterol metabolism. As for oxysterols, a better understanding of their roles can only be reached through their quantification in physiopathological situations.

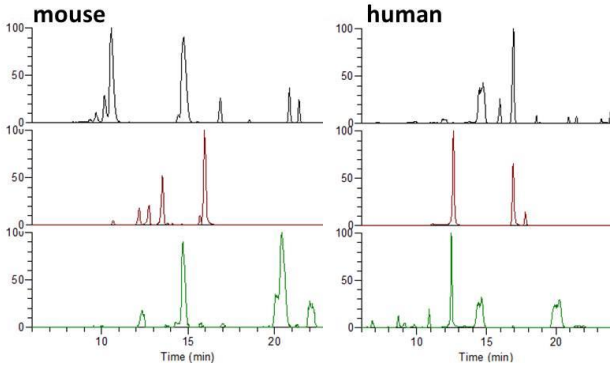

Figure 3: Representative EIC for the main bile acids present in mouse and human plasma. Analysis carried out by HPLC-MS using an LTQ-orbitrap operated in negative mode.

Thus this year, we developed an HPLC-MS method for the quantification of bile acids from several matrices, including mice liver and gallbladder content. Furthermore, to favor translational studies to humans, we adapted the method to quantify bile acids from serum/plasma samples (using either 30 µL of mouse serum or 50 µL of human serum) (Figure 3). Currently our method allows us to quantify 19 bile acids.

2. Study of the role of bioactive lipids in inflammation.

Numerous models (in vitro, ex vivo and in vivo) are used in the lab to study the role of bioactive lipid signaling in inflammatoty settings. For instance, we recently set up in vitro models (primary glial cells culture, CNS slices) to study the role of lipids on CNS inflammation. However in this year's report we put forth our work on colon inflammation.

We developed over the years an expertise in colitis murine models of colitis that mimic either the acute phase or the chronicisation of the disease that are both found in Crohn's disease and ulcerative colitis. (e.g. Alhouayek et al., FASEB J. 2011; Alhouayek et al., FASEB J. 2015)

Due to our long lasting interest in the endocannabinoid system (Muccioli, DDT, 2010; Alhouayek & Muccioli, TMM, 2012; Alhouayek*, Masquelier* et al. PNAS 2013), we noticed, using TNBS and DSS colitis models, that the levels of *N*-palmitoylethanolamine (PEA) were decreased in the inflamed colon. This observation, and the known anti-inflammatory effects of this bioactive lipid, prompted us to study the effects of increasing PEA levels during colon inflammation.

When administered to mice having either a TNBS-induced colitis or a DSS-induced colitis, PEA was able to reduce the colitis hallmarks in the colon, as well as central and peripheral inflammation.

Because we want to find potential pharmacological targets, we went on to determine which enzyme – FAAH or NAAA – actually controls PEA levels in the colon (Figure 4).

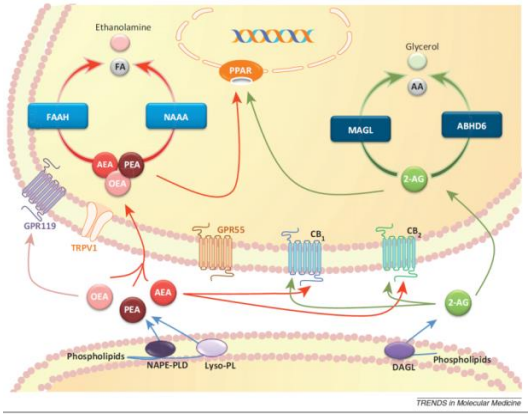

Figure 4: The endocannabinoid system in a nutshell (Alhouayek & Muccioli, TMM, 2012). The left part of the figure summarizes the metabolism and molecular targets of the N-acylethanolamines – anandamide (AEA), N-palmitoylethanolamine (PEA) and N-oleoylethanolamine (OEA). NAAA and FAAH are the two prime enzymes hydrolyzing the N-acylethanolamines.

We found that upon FAAH inhibition PEA levels are not increased in the colon and that FAAH inhibition does not recapitulate the effects of PEA when tested in TNBS-induced colitis. Inversely, NAAA inhibition increases PEA levels in the colon, and improves colitis (Alhouayek et al. FASEB J. 2015).

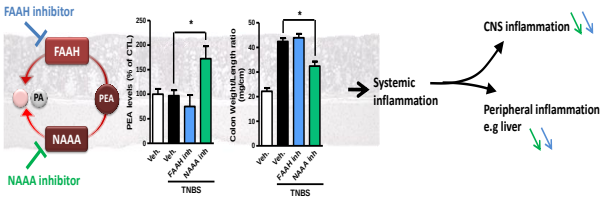

Figure 5: NAAA controls the colon levels of the anti-inflammatory bioactive lipid PEA. Inhibition of NAAA, but not FAAH, results in increased PEA levels in the colon. Increased PEA levels result in a reduction of all the measured hallmarks of colitis in the colon (e.g. the colon weight/length ratio shown here). NAAA inhibition, similarly to FAAH inhibition, also allows for a reduction in CNS and peripheral inflammation during colitis.

Thus modulating PEA endogenous levels, similarly to other bioactive lipids studied in the laboratory, allows for reducing the signs of colon inflammation (Alhouayek & Muccioli, TMM, 2012; Alhouayek et al., DDT, 2013) (Figure 5).

Because these acute models allow for the study of the most acute part of the disease, we also decided this year to set up a more chronic model of colitis.

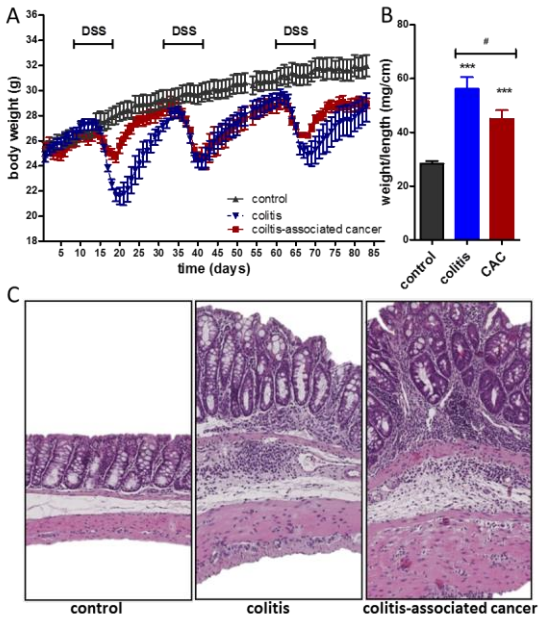

Figure 6: A. Evolution of the body weight of control mice and of mice having a chronic colitis (colitis) or cancer-associated colitis (CAC). B. Colon weight over length ratio for the same mice showing the presence of inflammation-induced thickening and shortening of the colon. C. Representative H&E photomicrographies of the mice colons.

This was achieved by administering to the mice repeated cycles of DSS (in the drinking water) followed by plain water (Figure 6). The succession of cycles mimics in mice the flares and remissions observed in Crohns' disease and ulcerative colitis. The adjunction of a pro-carcinogen results in a model of colitis-induced cancer. The roles of bioactive lipids in these two models are currently being studied in the lab.

In conclusion, the few examples described here of our current research clearly support the interest in developing both bioanalytical tools and pharmacological approaches in order **to increase our understanding of bioactive lipid signaling in inflammation and to put forth novel innovative therapeutic strategies**.

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Guillemot-Legris O, Mutemberezi V, Cani PD, Muccioli GG. Obesity is associated with changes in oxysterol metabolism and levels in mice liver, hypothalamus, adipose tissue and plasma. Sci Rep. (2016), 6:19694

Alhouayek M, Bottemanne P, Subramanian KV, Lambert DM, Makriyannis A, Cani PD, Muccioli GG. *N*-Acylethanolamine-hydrolyzing acid amidase inhibition increases colon N-palmitoylethanolamine levels and counteracts murine colitis. FASEB J (2015), 29(2):650-61.

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THESIS
In progress

Bottemanne Pauline "Study of the role of *N*-acylethanolamine-hydrolyzing acid amidase (NAAA) in inflammation and models of neurodegenerative diseases" Director: Giulio Muccioli

Buisseret Baptiste "Study of the effects of oxygenated derivatives of endocannabinoids in inflammation and inflammatory pain"

Director: Giulio Muccioli; Co-Director: Mireille Al Houayek

Caruano Josephine "Design, synthesis, and pharmacological evaluation of new Fatty Acid Amide Hydrolase inhibitors" Director: Raphaël Robiette; Co-Director: Giulio Muccioli

Guillemot-Legris Owein "Contribution to the study of the link between peripheral inflammation and neuroinflammation in metabolic diseases models" Director: Giulio Muccioli

Masquelier Julien "Contribution to the study of the central nervous system lipid composition in murine models of neuro-inflammatory and neuro-degenerative disease" Director: Giulio Muccioli

Mutemberezi Valentin "Setting up of quantification methods for oxysterols and oxysterols' metabolites: towards a better understanding of their role in inflammation" Director: Giulio Muccioli

Palmieri Vittoria "From inflammation to cancer: role of the endocannabinoid system in the development of IBD-associated colorectal cancer." Director: Giulio Muccioli, Co-Director: Mireille Al Houayek

Paquot Adrien "Development and validation of an HPLC-MS method to quantify the oxygenated derivatives of the endocannabinoids" Director: Giulio Muccioli

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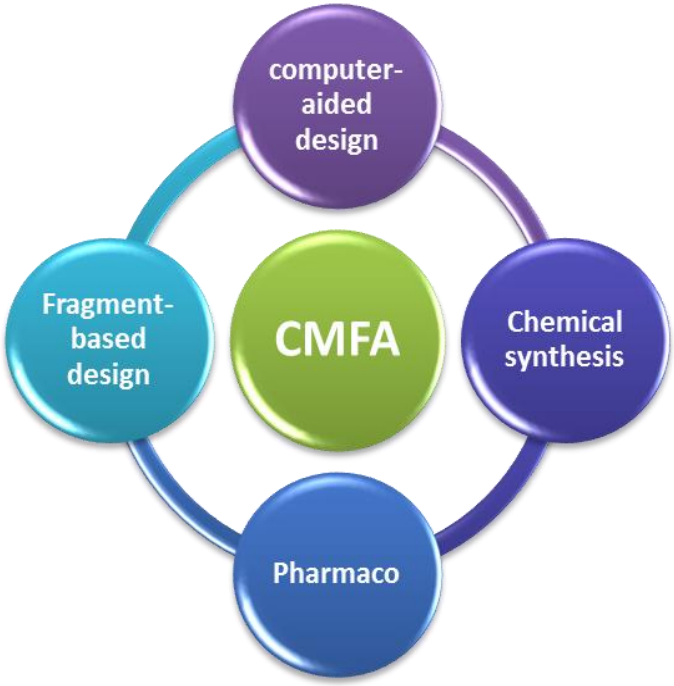
The discovery of new innovative medicines is a priority for human health. It is in this context that the Medicinal Chemistry Research Group (CMFA) is pursuing its research activities

To reach this ambitious objective, two complementary strategies are used:

The first is driven by a pharmacological approach aiming at designing new bioactive molecules mainly interacting with a physiological system of neurotransmission: the endocannabinoid system. This system consists of several proteins regulating the signaling of endogenous lipid compounds, i.e. the endocannabinoids. Several targets emerge from this system: endocannabinoid biosynthesis enzymes, GPCR receptors, nuclear receptors and endocannabinoid degradation enzymes.

The second complementary approach is driven by a chemical approach of drug design and aims at discovering new chemical entities following a computationally-assisted strategy. This strategy involves the initial discovery of hits via virtual screening and fragment-based inspired methodology. These hits are then optimized by rational drug design to generate new pharmacological tools or drugs. Today our researches are mainly focused on the search of new anticancer agents, and particularly for the discovery of new agents for anticancer immunotherapy.

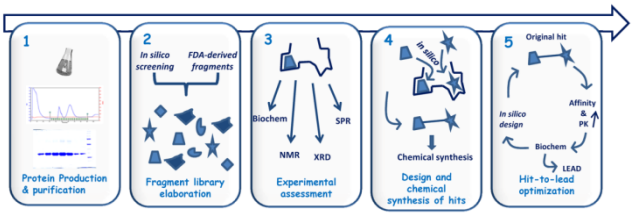


RESEARCH RESULTS

Fragment-based drug design

In medicinal chemistry, one of the big challenges remains the discovery of an original hit that can be easily tuned into a lead and then in a drug candidate. In this regard, our aim is to develop an innovative fragment-to-lead strategy for high-quality lead identification. This computationally-assisted approach involves the initial discovery of low-molecular weight molecules called fragments. Owing to their small-size, fragments are more likely to reach key pockets within a protein active site, and, once their interaction within the active site is clearly understood, they represent a unique possibility of designing a promising hit compound in an efficient way.

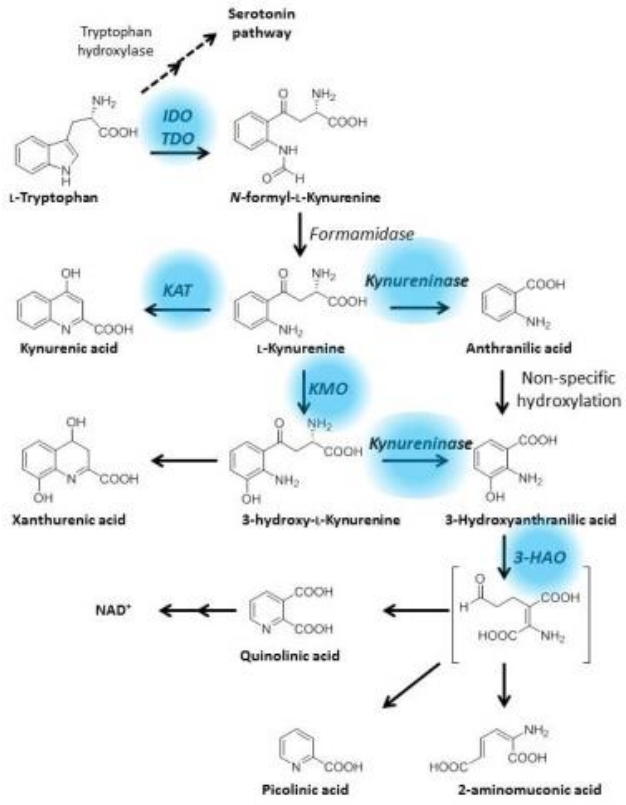
The originality of this approach resides in the compilation of available experimental information about structural motifs recognized by the target, and their use to guide the selection of fragments from large chemical databases using a computationally-assisted screening. The interaction of chosen fragments with the target protein can then be experimentally assessed by means of biochemical and biophysical techniques, such as NMR, surface plasmon resonance (SPR), mass spectrometry (MS) and/or X-ray diffraction (XRD). Once the binding experimentally confirmed, rational drug design can start and the selected fragments can be finely tuned to provide an original hit. This original computationally-assisted fragment-to-lead strategy offers the prospect of a more efficient approach to drug discovery – resulting in the generation of high-quality leads with a better chance of success in future development.



Anticancer immunotherapy

Tryptophan catabolism is an important mechanism of peripheral immune tolerance contributing to tumoral immune resistance, and indoleamine 2,3-dioxygenases (IDO and TDO) inhibition is a promising strategy for anticancer drug development. IDO and TDO are unrelated heme-containing enzymes catalyzing the oxidative cleavage of the indole ring of L-tryptophan (L-Trp), the first and rate-limiting step along the kynurenine pathway. The implication of IDO in the phenomenon of tumoral immune resistance is the focus of intense researches and the enzyme is now recognized as a validated target for anti-cancer therapy. Therefore, a number of groups, including us, are actively searching for novel original IDO inhibitors. In contrast, the effect of TDO expression on the immune response has only been recently investigated in detail. Indeed, we showed in collaboration with the group of Prof Van den Eynde that TDO was effectively overexpressed by a number of human tumors and that this expression prevented rejection of tumor cells. We designed a novel TDO inhibitor and proved, in a preclinical model, the concept that TDO inhibition promotes tumoral immune rejection. Interestingly, blocking both TDO and IDO to improve the efficacy of cancer immunotherapy would be complementary: in a series of 104 human tumor lines of various histological types, we showed that 20 tumors expressed only TDO, 17 only IDO and 16 expressed both enzymes. Therefore,

targeting both IDO and TDO would allow reaching 51% of tumors instead of either 32% or 35% with a compound inhibiting IDO or TDO alone, respectively. The design of IDO, TDO or dual IDO/TDO inhibitors is thus of major importance. Interestingly, our fragment-based drug design strategy recently provided promising results for the discovery of new IDO. These preliminary data are very encouraging to pursue the search for new anticancer agents through a fragment-based drug design strategy.

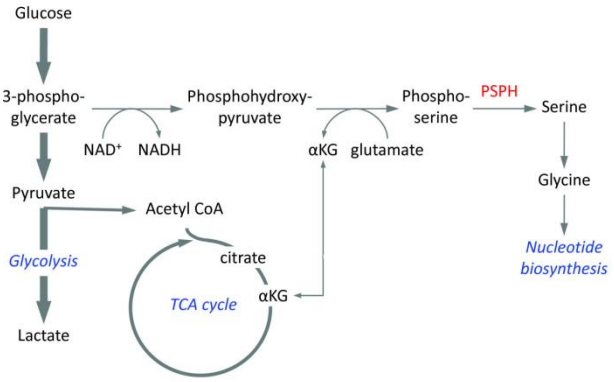


Tumor metabolism

This project aims to understand the role of the serine pathway in tumor progression and in particular to develop pharmacological tools to evaluate the extent of tumor addiction to this metabolic path and their therapeutic potential by exploring potential side effects on healthy tissues. To this end, novel innovative pharmacological inhibitors of PHGDH and PSAT1, the two main enzymes of the serine pathway (see Figure 1), will be designed and chemically synthesized. These compounds will help deciphering the exact roles of these enzymes in cancer progression and will provide insights on their physiological roles (that could represent limitations to the clinical use of such inhibitors).

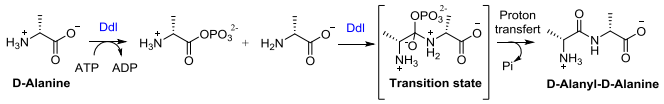
The last five years have witnessed an increased regain of interest for tumor metabolism. Recent advances in this field have shed light on how tumors fuel rapid growth by preferentially engaging biosynthetic pathways. Although cellular metabolic pathways are rich pickings for drug targets, pinpointing enzymes that critically contribute to tumor metabolism is key to establish a therapeutic window since most of metabolic enzymes also play important roles in normal tissues. PHGDH (3-phosphoglycerate dehydrogenase) and PSAT1 (phosphoserine aminotransferase-1) could however represent such ideal targets for new anticancer strategies. These enzymes catalyze the first and second steps in the serine biosynthetic pathway, respectively. This pathway diverts a relatively small fraction of 3-phosphoglycerate from glycolysis to generate serine as well as equimolar amounts of NADH and α-ketoglutarate (αKG). Interestingly, two simultaneous recent reports have recently identified the serine pathway as a vital source of αKG to fuel the TCA cycle in a variety of tumor cells. These two studies further documented that serine supplement could not rescue tumor cells in which PHGDH and PSAT1 genes were knocked down, thereby identifying the

serine pathway as a process providing malignant cells with critical amounts of its intermediary synthetic products, αKG and possibly NADH, instead of the end product, serine (that may also be taken up from the extracellular fluid). In good agreement with the above statement on the rationale to identify specific targets to tackle tumor metabolism, this latter observation indicates that serine deficiency in healthy tissues and possible disorders associated with the inhibition of either PHGDH or PSAT1 could be treated by exogenous serine supplement, whereas treatment with such inhibitory compounds could take advantage of the strict addiction of tumors to the by-products resulting from PHGDH and PSAT1 activation.



Antibiotics

As the phenomenon of antibiotic resistance is dramatically increasing these days, the search for new therapeutic targets less vulnerable to these resistance mechanisms appears as a real need. The cell wall of bacteria and the enzymes that are involved in its synthesis are prime targets for many antibiotics, which inhibit the late stages of peptidoglycan biosynthetic pathway. But the resistance phenomena have revealed the high flexibility in this assembly pathway, and the need to target other enzymes acting on earlier steps of peptidoglycan synthesis. D-alanyl-D-alanine ligase (Ddl) is of particular interest as it utilizes a substrate (D-alanine) which is specific for bacterial peptidoglycan biosynthesis and essential for bacterial growth.



In this work, we aim at designing novel DD-ligases inhibitors. Previous works in our group have highlighted a novel hit (S89) characterized with thiosemicarbazide motif. First, analogues of S89 were synthesized. Indeed, the thiosemicarbazide family is very promising due to its low half maximal effective concentration (EC50) and its good antibacterial activity. These compounds will be evaluated on recombinant protein Ddl-His6 produced and purified in our group. This study will provide initial structure-activity relationships (SAR) and thus help understanding the structure requirements to achieve a high DD-ligases inhibition. Then, novel hits will be identified through a fragment-based strategy. To this end, an in-house library of 280 diverse fragments will be first assessed. Finally, the more potent fragments will undergo a structure guided optimization to design potent DD-ligases inhibitors.

Cannabinoid system modulators

The aim of this work is to design new bioactive molecules interacting with a physiological system of neurotransmission: the endocannabinoid system. This system consists in several proteins regulating the signaling of endogenous lipid compounds,

i.e. the endocannabinoids. Several targets emerge from this system: endocannabinoid biosynthesis enzymes, GPCR receptors, nuclear receptors and endocannabinoid degradation enzymes.

Ongoing research involves the development of selective inhibitors for the three main enzymes involved in the degradation of the endocannabinoids, namely the fatty acid amide hydrolase (type I), the monoacylglycerollipase, the N-acylethanolamine Acid Amidase. Several inhibitors of endocannabinoids metabolism have been discovered. The main achievement was the synthesis of the first inhibitor of the N-acylethanolamine Acid Amidase. Regarding monoacylglycerollipase (MGL), the search was focused on selective inhibitors, disulfiram has been identified as MGL inhibitor with a high selectivity profile regarding fatty acid amide hydrolase (type I).

Trypanosomiasis chemotherapeutics.

Infections caused by trypanosomes remain a severe health problem in Western Africa. Some treatments are available. However, the useful drugs are rather expensive and induce severe side-effects. There is therefore an urgent need to provide the medical community with new improved drugs. Along this line, we launched a medicinal chemistry research program using acetophenone thiosemicarbazone as lead compound. Initial work was devoted to getting access to a flexible and "green" access route to these target compounds by development of an efficient catalysis. After having synthesized a compound library of some 150 terms, we ended up with compounds with IC50's in the sub-micromolar range endowed with a rather good safety index (~100). Further work is now being performed to expand the druggability within this series.

Besides our main research topics, we are offering our technological chemical and pharmacological expertise in to other research scientists from UCL or external groups.

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Didier LAMBERT

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THESIS
In progress

Ameryckx Alice "Design and synthesis of DD-ligases inhibitors: Peptidoglycan intracellular biosynthesis as antibiotics target" Director: Raphaël Frédérick; Co-director: Françoise Van Bambeke

Julien Prévost "Design and synthesis of Arg1 inhibitors for anticancer immunotherapy" Director: Raphaël Frédérick

Quentin Spillier "Towards the understanding of the role of the serine pathway in tumor progression" Director: Raphaël Frédérick; Co-director: Olivier Feron

Comlan Urbain Kassehin "Synthesis and Pharmacological Evaluation of Trypanocidal Arylthiosemicarbazone Derivatives" Director: Jacques Poupaert; Co-director: Joëlle Leclercq

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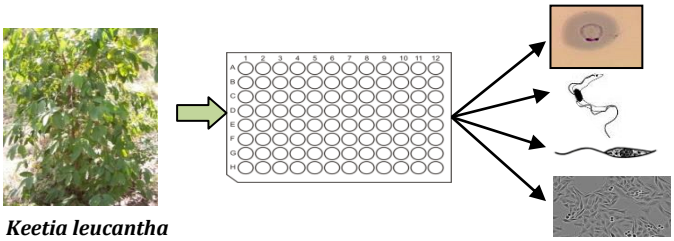
Pharmacognosy *implies multidisciplinary studies to identify new drug candidates (pure compounds or extracts) or new leads from natural origin and control their quality.*
Our laboratory, created in 1996, chose to focus on plants used in traditional medicine to:

1. *Evaluate the activities of crude extracts from traditional medicinal plants and obtain data to support their traditional uses, their indications and analyse potential toxicities.*
2. *Isolate and identify bioactive compounds which could constitute new prototypes for drug development*
3. *Analyse the possible targets and identify structure-activity relationships*
4. *Control their quality to limit adulterations and standardise treatments.*

To allow these researches, we developed an expertise in purification, structure determination of compounds from complex matrices and development of quantification validated methods, while most of the pharmacological experiments are realised in collaboration with teams having expertise in the selected biological activities.
Our future researches will mainly focus on antiparasitic, antimicrobial and anticancer activities for which a majority of available drugs are natural substances or derivatives.

1/ CRUDE EXTRACTS AND PURE COMPOUNDS EVALUATIONS

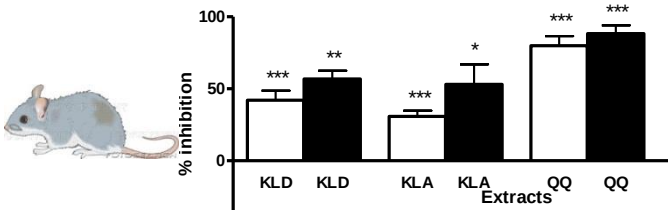
Plants used in traditional medicine in different countries are obtained through research collaborations (Marocco, Benin, Congo Democratic Republic, Rwanda, Madagascar, Mauritius in Africa, Vietnam in Asia, Peru, Bolivia and Brazil in South America). The first step is the selection on an ethnopharmacological basis and a literature survey. Different extracts are prepared and pharmacologically evaluated according to their traditional use(s). Several properties are analysed in collaboration with other teams who developed suitable pharmacological tests (LDRI, other UCL or Belgian partners): antimicrobial, cytotoxic, immunostimulating, anti-angiogenic, anti-inflammatory, antihypertensive, anti-coagulant, antioxidant and antiplasmodial activities, as well as inhibition of A-Beta formation, while tests for antitrypanosomal, cytotoxic and antileishmanial properties are now developed in the lab.
Crude extracts are first evaluated by *in vitro* tests and their cytoxicity assessed on cancer and non-cancer cell lines.



Keetia leucantha

The originality of our works is that we do not just realise screenings. The most promising extracts are also tested *in vivo* to assess their activity and eventual toxicity. The mode of administration is chosen according to the nature of the extract but most of them are given by oral route.
Several extracts possessing biological activities *in vitro* were identified (cfr publications).
The activities of the most interesting ones as well as purified compounds were also analysed *in vivo*. Results indicate that extracts of *Croton zambesicus* and *Marrubium vulgare* showed, *in vivo*, antihypertensive properties but some extracts of *Croton zambesicus* also showed toxicities. Extracts from i.e. *Keetia leucantha*

and *Acanthospermum hispidum* as well as isolated triterpenic esters proved to have antimalarial activities on mice infected by *Plasmodium berghei.* Efficient extracts on mice infected with *Trypanosoma brucei* were also identified and their highest tolerated dose determined.

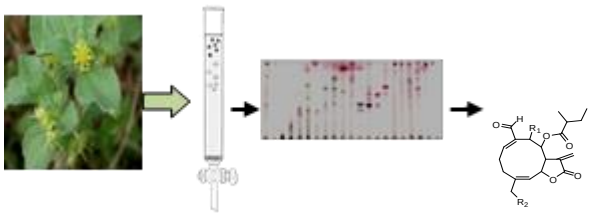


Our researches also led us to identify further *in vivo* toxic compounds from nutrient rich seeds of *Pachyrhizus* species, consumed in certain parts of Asia.

Our research on antimicrobial plants allowed us to identify some promosing plant extracts and natural compounds reducing the resistance of methicillin resistant *Staphylococcus aureus* (collaboration with F. Van Bambeke).

2/ ISOLATION AND STRUCTURE IDENTIFICATION OF BIOACTIVE NATURAL COMPOUNDS

Plant extracts having interesting *in vitro* and/or *in vivo* activities are subject to bio- and chimio- guided fractionations to identify active components which could constitute new leads for further developments.



Acanthospermum hispidum

Fractions obtained by different chromatographic methods are evaluated and active ones analysed by LC-MS to identify well known compounds (based on retention times and MS^n spectra, collaboration with MASSMET platform) and determine those which should be further purified (unidentified substances). Structural identification is based on UV, IR, SM, 1D and 2DNMR spectra.

In addition to known compounds, we identified several new molecules which are found for the first time in plants. Among them, we can point out diterpenes isolated from *Croton zambesicus*. Some of these diterpenes have been shown by our team to possess cytotoxic and pro-apoptotic properties but others relax significantly rat aorta contracted by KCl. Comparison of the cytotoxic and vasorelaxant activities of isolated molecules and synthetic analogues indicates that both effects are not linked. We can also cite several promising specific antiparasitic terpenic derivatives isolated form *Keetia leucantha*, *Ocimum basilicum* or *Cymbopogon* species.

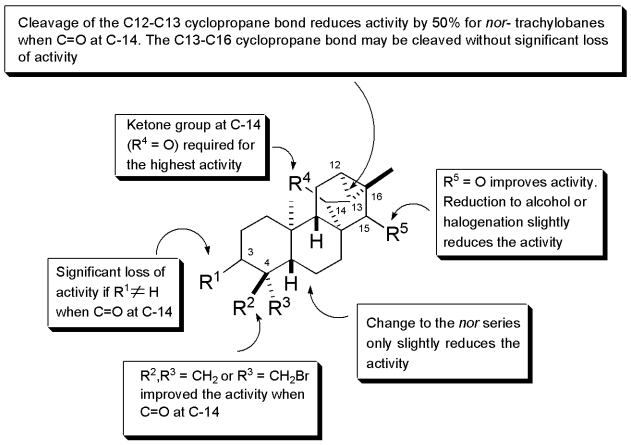
Identifications of antimalarial compounds is also guided by supervised metabolomics studies of crude extracts.

3/ IDENTIFICATION OF TARGET(S) AND STRUCTURE-ACTIVITY RELATIONSHIPS

Once structures are identified, we realised further experiments in collaboration with specialised teams to determine their targets and modes of actions and compare their activities with related natural or (semi)-synthetic compounds to assess structure-activity relationships.

We also analysed the possible targets for crude extracts. For example the activity of an extract of *Keetia leucantha* on different forms of trypanosomas showed a possible effect on glycolysis. We also proved the

inhibiting effect of *Pterocarpus erinaceus* extracts on γ-secretase, an enzymatic complex responsible for A-Beta formation, and the effect of *Croton zambesicus* or *Marrubium vulgare* extracts on voltage dependent calcium channels.
Researches on pure isolated compounds allowed us to determine some structure-activity relationships for the vasorelaxant effect of trachylobanes diterpenes (collaboration with N. Morel, IREC). Targets were identified as voltage dependent calcium channels.



Structure-activity relationships for the vasorelaxant activity of trachylobanes

Alkaloids inhibiting topoisomerase I were identified in *Cassytha filiformis*. Synthetic derivatives were prepared in Spain and were also shown to possess antimalarial properties with a high selectivity index. Structure-activity relationships have been studied.

In the antiparasitic domain, we identified several antitrypanosomal terpenic compounds, some of them inhibiting trypanosomal GAPDH activity, a key enzyme of glycolysis, a process vital for trypanosoma development during its human cycle. We also collaborate with the teams of Prof. J. Palermo (University of Buenos Aires), Profs. J. Poupaert and R. Frédérick (LDRI-CMFA) and Profs. G. Acrombessi and F. Gbaguidi (UAC-Bénin) for the evaluation of the antiparasitic activities of (semi)synthetic compounds and establishment of structure-activity relationships.
The physico-chemical interactions of natural saponins with cholesterol and biological membranes were studied in collaboration with the team of M.P. Mingeot (TFAR-FACM/LDRI) and new results were obtained which could explain several activities of this class of compounds.

4/ QUALITY CONTROL AND ANALYTICAL VALIDATED METHODS DEVELOPMENT

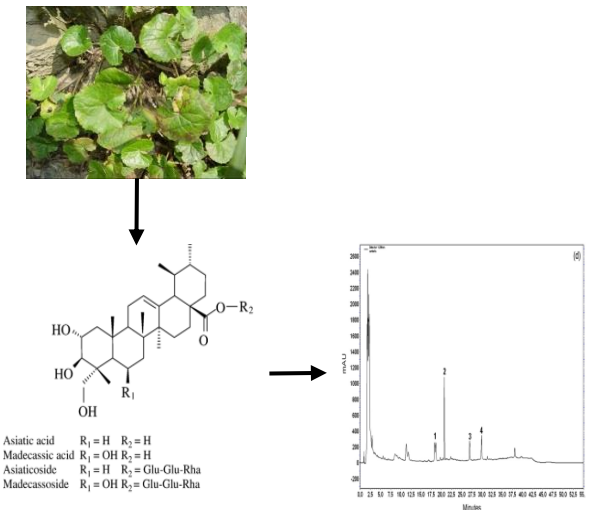
The last part of our research is to develop and validate analytical methods to identify and quantify natural compounds in complex media (crude extracts, cells, biological fluids…).

Analytical methods are useful:
- To control the quality of plant preparations

- To increase the yields and/or the quality of productions by studying the effects of growth/cultivating/harvesting conditions on the active molecules contents of plants.

- To analyse the mode of action, resorption and/or metabolisation of natural substances or derivatives

- To find methods to eliminate toxic compounds and find less toxic accessions.



Methods to identify by LC-MS and quantify several types of bio active molecules by GC-FID, GC-MS, LC-UV or LC-MS in crude extracts (particularly alkaloids, mono-, di-, triterpenes, steroids, rotenoids and flavonoids) were developed and validated in collaboration, for LC-MS, with MASSMET platform. We also developed a model to predict and analyse metabolic stability, identify metabolites from pure compounds and quantify anti-angiogenic hemi-synthethic products in blood.
The laboratory is also officially agreed (by the Federal Agency for Medicine and Health Products) for the quality control of drugs.

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Bero J., Beaufay C., Hannaert V., Hérent M.F., Michels P.A., Quetin-Leclercq J. Anti-trypanosomal compounds from the essential oil and extracts of *Keetia leucantha* leaves with inhibitor activity on *Trypanosoma brucei* glyceraldehyde-3-phosphate dehydrogenase. Phytomedicine (2013), 20: 270-274.

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THESIS
In progress

Beaufay Claire "Evaluation des activités antiparasitaires de plantes utilisées en médecine traditionnelle" Directors: Joëlle Quetin-Leclercq, Joanne Bero.

Catteau Lucy "Recherche de nouvelles molécules d'origine naturelle ayant des propriétés antimicrobiennes ou inversant la résistance aux antibiotiques" Directors: Joëlle Quetin-Leclercq, Françoise Van Bambeke.

Comlan Kassehin Urbain** "Conception, synthèse et évaluation pharmacologique de thiosemicarbazones en tant qu'anti-bactériens, antiparasitaires et antiviraux" Directors: Jacques Poupaert, Fernand Gbaguidi, Joëlle Quetin-Leclercq.

Sounouvou Hope** "Etude des potentialités de plantes à huiles essentielles béninoises pour le traitement d'infections cutanées et mise au point de formulations topiques" Directors: Joëlle Quetin-Leclercq, Brigitte Evrard, Fernand Gbaguidi.

Toukourou Habib** "Etude de l'innocuité et des potentialités d'huiles essentielles pour le traitement d'infections respiratoires" Directors: Joëlle Quetin-Leclercq, Fernand Gbaguidi.

** « Mixed » doctorate – Defensis at the UAC (Université d'Abomey-Calavi) –Experimental work done mainly in Belgium

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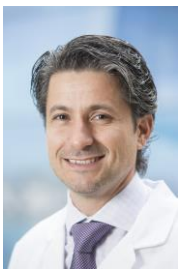

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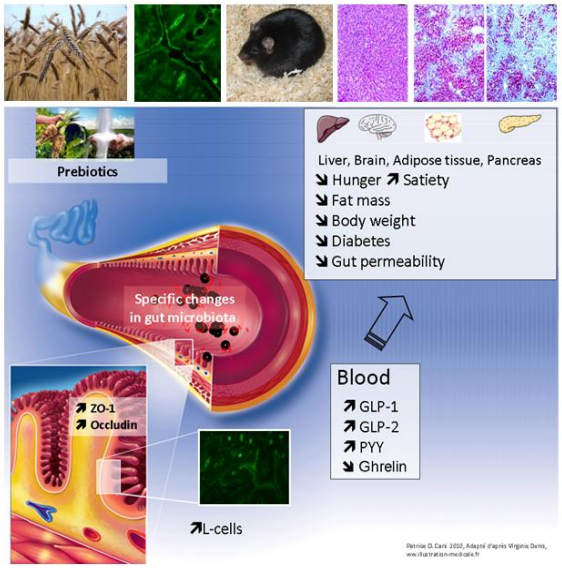
Our research group propose an approach based on integrative physiology, metabolism and nutrition, in order to investigate the role of the gut microbiota in the development of metabolic disorders associated with obesity (metabolic inflammation, insulin resistance, type 2 diabetes, cardiovascular diseases), malnutrition (cachexia associated with tumor development, nutrient deficiencies, ageing, alcohol consumption....) or changes in cellular homeostasis leading to cancer (e.g. tumor development).
Our scientific activities are mostly focusing on nutrients targeting the gut microbiota, such as carbohydrates which escape the digestion and which are largely fermented in the colon by specific bacteria -called prebiotics- as well as to understand the molecular mechanisms linking gut microbes to the host metabolism. The development of functional food targeting the gut appears as an interesting way to modulate key metabolic functions in the body, in order to improve health and well-being.

One main issue is to characterize the impact of nutrition and diseases on specific molecular targets such as gut endocrine and barrier functions, the endocannabinoid system, the innate immune system in the control of body weight, fat mass, systemic immunity and energy homeostasis. Our purpose is to elucidate the contribution of the the gut microbiota in the control of those systems.

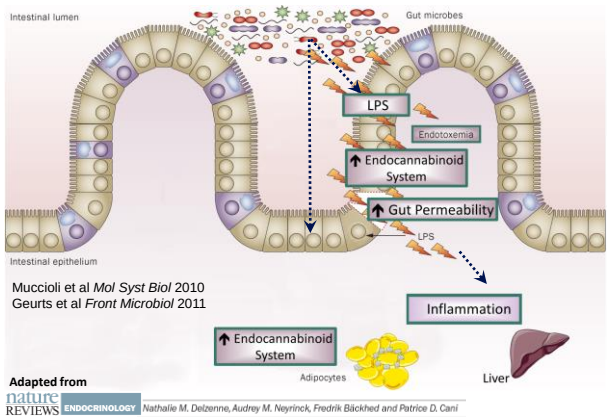


Models and experimental approaches

Experimental animal models (through genetic, pharmacologic or nutritional manipulation) and a panel of biomarkers and techniques have been developed in order to assess the molecular mechanism underlying the "metabolic bridge" built by the gut microbiota between the colon and key organs involved in the control of energy metabolism (brain, liver and adipose tissues, muscle).
Furthermore, specific *in vitro* models, such as "Precision-Cut Liver Slices (PCLS)", have been adapted to study the contribution of tissue-fixed macrophages in the metabolic response to nutrients and drugs. In addition, a panel of cancer cell lines obtained from a variety human tissues (i.e. liver, breast, haematological malignancies, brain...), allowing us to perform *in vitro* studies characterizing the impact of both inflammatory and oxidative stresses on cancer cell metabolism.
Finally, the integrative physiology of the different metabolic systems (including

microbiota) is studied by *in vivo* experiments in live animals, and by (meta)genomic and metabolomics approaches in biological fluids and tissues, performed in collaboration with key specialists in the field. Nutritional intervention studies are also performed in humans in collaboration with colleagues of St Luc hospital or from abroad.

One of our recent breakthroughs has been the identification of the role of the endocannabinoïd system and its interaction with the gut microbiota in the development of adipose tissue and metabolic inflammation associated with obesity, insulin resistance and type 2 diabetes.



To this aim, specific animal models of tissue specific genetic deletions of genes involved in the host-bacteria interaction or in the synthesis of endocannabinoids are currently developed and studied. In addition, both *in vivo* and *in vitro* models are proposed to analyse the modulation of metabolic, oxidative, and inflammatory stresses by nutrients, ingredients and/or pharmacological compounds.

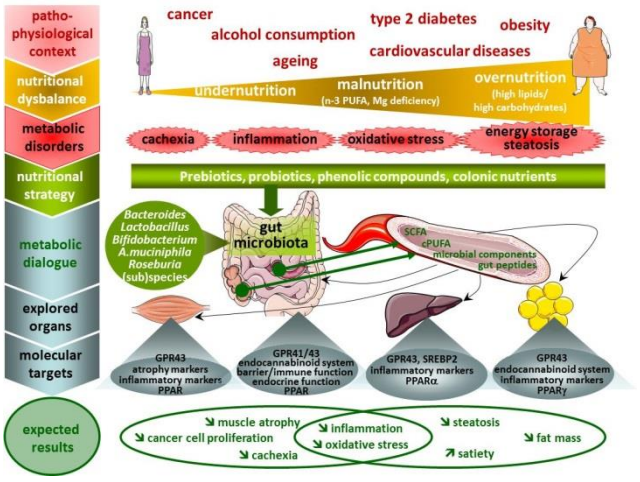
RESEARCH RESULTS

The main research activities performed upon the last five years were devoted:

1. to develop experimental models mimicking metabolic disturbances occurring upon obesity and cancer development,
2. to test for the implication of gut microbiota in the occurrence of those metabolic disorders,
3. to investigate the role of the endocannabinoid system and or of specific receptors responding to gut microbial components or metabolites ,
4. to decipher the role of the innate immune system in the development of obesity, inflammation, insulin resistance, oxidative stress, type 2 diabetes, hepatic steatosis and cancer cachexia in mice.
5. to evaluate the involvement of key gut functions (endocrine, immune, endothelial, barrier functions) alterations in the occurrence of behavioural and metabolic disorders.
6. to explore the potential links between oxidative stress, inflammation and cancer cell metabolism.

Those alterations are namely dependent on the gut microbiota and specific bacterial derived compounds such as pathogen-associated molecular patterns (PAMP's). Among them, we have identified the key role played by the lipopolysaccharides (LPS) in the onset of metabolic inflammation and glucose homeostasis disorders in the context of obesity and associated disorders, as well as in the inflammation linked to alcohol dependence in humans. The alteration of the gut barrier is one important cause of the translocation of bacterial elements (LPS, peptidoglycans..) which promote inflammation and metabolic disorders occurring in nutritional or behavioural disorders (diabetes and obesity, cancer cachexia, alcohol dependence…) (For review Delzenne et al Diabetologia 2015, Cani and Everard Molecular Nutrition Food Research 2016).

There is a link between the composition of the gut microbiota – that is profoundly modified in genetic (*ob/ob*) and dietary models of obesity - and the control of body weight, insulin secretion/response, inflammation and appetite. The gut microbiota could also be involved in the hepatic steatosis and vascular disorders induced by nutritional deficiency in essential polyunsaturated fatty acids, as well as in the occurrence of cachexia and inflammation linked to systemic cancer development. Non digestible carbohydrates such as inulin-type fructans are defined as prebiotics since they are highly and selectively fermented by certain bacterial species and thereby improve host health. We have tested the influence of several non-digestible dietary carbohydrates (e.g., fructans, cereal subfractions, and/or glucans derivatives, pectooligocaccharides…) on gut fermentation and systemic metabolism.



Our experimental data suggest their potential to improve metabolic disorders associated with obesity (insulin sensitivity, immunomodulation, fat mass development). In rodents and humans, changing the gut microbiota composition by using fructans improves glucose homeostasis, steatosis and decrease fat mass development, and these events being clearly related to the modulation of endogenous gut peptides production. The gut microbiota participate to specific intestinal metabolic responses, for instance, changing the microbiota with dietary prebiotics administration leads to an increase in the differentiation of stem cells into endocrine L cells in the proximal colon of rats, and therefore promotes the production of glucagon-like peptide-1 and 2 (GLP-1 and GLP-2) in this organ. The relevance of the GLP-1 in the improvement of metabolic disorders is shown through experiments performed in mice lacking functional GLP-1 receptor: those mice are resistant to the beneficial effect of fructans on obesity and glucose metabolism. In addition, the GLP-2 is known to improve gut barrier function, here we found that the endogenous production of GLP-2 is a key event responsible for the reduced gut permeability observed upon severe obesity and type 2 diabetes. Recent data obtained following high-throughput molecular analysis of bacterial 16S rRNA allowed to point out novel interesting bacteria (Bifidobacteria, *Akkermansia muciniphila*, *Roseburia spp., Lactobacillus spp.,* …) or yeast (*Saccharomyces boulardii*) in the control of host hormonal status, adiposity, and immunity.

Overview of the recent result:
In the context of cardiometabolic disorders

In 2013, we have identified *Akkermansia muciniphila* as a key bacteria involved in the control of the gut barrier function and host metabolism (Everard et al PNAS 2013). We demonstrated that *A.muciniphila*, a mucin-degrading bacteria that resides in the mucus layer and abundantly colonizes it, negatively correlates with body weight and is decreased under high-fat diet. Moreover, daily administration of *A. muciniphila* to high-fat-diet-induced obese mice for 4

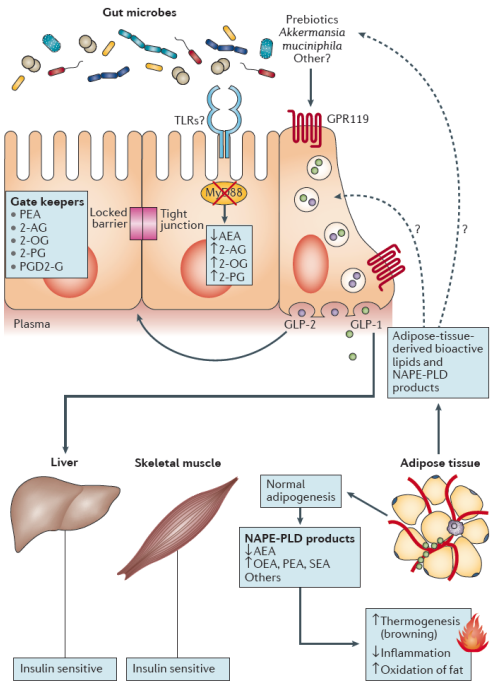
weeks improves metabolic profile, by decreasing weight gain, restoring mucus layer thickness, antimicrobial peptides (Reg3gamma) production and counteracting metabolic endotoxemia and insulin resistance.
Our studies have also indicated that the gut microbiota clearly participate to the control of metabolic endotoxemia and of the release of proinflammatory cytokines (IL1, IL6, MCP1…) in both nutritional and genetic models of obesity.

We discovered that the fatty acids composition may also strongly contribute to the modulation of the abundance of *Akkermansia muciniphila*. We found that mice fed with a saturated fatty acid diet (lard-enriched diet) exhibited a significant decrease in *Akkermansia muciniphila*, whereas omega 3 fatty acids (fish oil-enriched diet) dramatically increased *Akkermansia muciniphila* in the gut. This effect was associated with a better gut barrier function and decreased adipose tissue inflammation, a phenomenon that can be transferred to germ-free recipient mice (Caesar et al. Cell Metabolism 2015).

We have also revealed a link between *Akkermansia muciniphila* and age, since the intestinal levels of this bacterium declined with age upon a normal diet feeding. We found that high-fat diet feeding strongly influenced adipose tissue profile and intestinal microbiota in a way that mimicked aging, or at least older mice. In the same set of experiments, we found by using multifactorial analysis that these changes in *A. muciniphila* were robustly linked with the expression of lipid metabolism and inflammation markers in adipose tissue, as well as several blood markers (i.e., glucose, insulin, triglycerides, leptin) (Schneeberger et al Sci Reports 2015).

In obese humans, in accordance with the data obtained in rodents, we found that in the basal state, the abundance of *Akkermansia muciniphila* is inversely related to fasting glucose levels, visceral fat accumulation, and adipocyte diameter in subcutaneous adipose tissue. In addition, upon caloric restriction, obese individuals with higher baseline *Akkermansia muciniphila* exhibited a greater improved insulin sensitivity as well as an improvement of different markers and other cardiometabolic risk factors (e.g., plasma cholesterol, inflammation) (Dao, Everard et al. GUT 2016).

Thus, all these data suggests that increasing the intestinal levels with nutrients or the administration of *Akkermansia muciniphila* is of interest and merit further investigation in humans. The team of Prof. Cani is currently investigating this question in obese patients, study started in December 2015 (www.microbes4U.be).



*(Patrice D. Cani, Hubert Plovier, Matthias Van Hul, Lucie Geurts, Nathalie M. Delzenne, Céline Druart and Amandine Everard. **Nature Reviews Endocrinology dec 2016**).*

We have previously identified that the endocannabinoid system links the gut microbiota to adipogenesis in both physiological and pathological situations such as obesity and type 2 diabetes. Our novel data pointed out that targeting specifically the endocannabinoid system tone in the adipose tissue may contribute to change host-microbiota interactions (for review Cani et al Nature Reviews Endocrinology 2016). In 2015, we published data showing that deleting NAPE-PLD in the adipose tissue (tissue specific deletion) induces obesity in normal diet-fed mice by promoting fat mass development, insulin resistance and inflammation. We discovered that the deletion of NAPE-PLD in adipocytes induces also a decreased thermogenic programme (i.e., browning/beiging) in adipose tissue. Importantly, we found that NAPE-PLD deletion in adipose tissue induced a profound shift in the gut microbiota compositon and activity.

Finally, we have proven that the microbiota contribute to the phenotype. By transferring the microbiota from mice in which the adipose tissue NAPE-PLD was deleted into germ-free recipient mice replicated the overall phenotype. Taken together, these findings indicate that bioactive lipids produced by adipose tissue contribute to changes in the gut microbiota; these changes then participate in the altered metabolic disorders observed following NAPE-PLD deletion (Geurts et al. Nature Communications 2015). These results provide strong support for the crosstalk between adipose tissue and gut microbiota, with the endocannabinoid system as a potent mediator.

In 2014, we found that a link between the innate immune system from intestinal cells (i.e., the protein MyD88) and energy homeostasis. More precisely, we found that modifying the response of the immune system by deactivating the protein MyD88 in the intestinal cells delay the development of type 2 diabetes induced by a high fat diet, reduces the development of fat mass, reduces the deleterious inflammation observed during obesity and reinforced the gut barrier thereby preventing the leakage of unsuitable bacterial compounds from the intestine to the organism. More importantly, we found that it is experimentally possible, through this modification of the immune system, to induce body weight loss and therefore to have a therapeutic effect despite the fact that the animals were already obese and diabetic. Surprisingly, we found that it is possible to partially protects against obesity and diabetes by transferring (i.e., grafting) the gut microbiota from these mice to axenic mice (i.e., germ free) (Everard et al. Nature Communications 2014). We are currently investigating the role of Myd88 deletion in the hepatocyte and host metabolism (Duparc et al, GUT 2016).

Overview of the recent result:
In the context of cancer cachexia

In different mice models of cancer cachexia, we could establish a link between specific changes in gut microbiota composition/function and host metabolism. A drop in bacterial diversity and of specific Lactobacilli -which are prone to exert anti-inflammatory properties-, or a bloom in Enterobacteriacaea, are related to changes in gut barrier function, muscle atrophia and inflammation in cancer bearing mice. Those changes are not the consequence of a decrease in food or nutrients intake, but can be related to perturbations of the secretion of anti-microbial peptides and of the immune system in the gut epithelium. The administration of selected viable bacteria (*Lactobacilli reuteri*), as well as the dietary supplementation with various non digestible oligo-saccharides with prebiotic properties

(inulin, pecto-oligosaccharides) can be efficient in reversing some dysbiotic patterns and improving host metabolism in this context. Dietary prebiotics prone to release propionate upon fermentation could also play a role in the control of cancer cell proliferation in the liver.

Our data support that metabolites produced by bacteria from dietary lipids (conjugated polyunsaturated fatty acids, bile acids…) from carbohydrates (lactate, propionate, butyrate..), or from amino acids (phenol or indol derivatives) could also be important mediators allowing to establish a metabolic dialogue between the gut microbiota and host organs (liver, adipose tissue, muscle), in different context of nutritional disturbances leading to metabolic, oxidative and inflammatory stresses. Interestingly, oxidative stress and inflammation are mostly mediated by signalling pathways where Nrf2 (a pleiotropic transcription factor involved in cellular defences) plays a major role. In this context, we have shown that interfering the activation of Nrf2 in cancer cells will render them sensitive and vulnerable to a pro-oxidant therapy.

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Nathalie DELZENNE

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Pedro BUC CALDERON

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AWARDS 2014-2015

Bindels Laure
Young investigator award at the 8th Cachexia Conference

Cani Patrice D.
Baillet Latour grant for Medical Research 2015

Cani Patrice D.
Officer of the Walloon Merit (O.M.W.) (2015)

Cani Patrice D.
Laureate of the prize Transatlantic Bank of Belgium (St Luc foundation) (2015)

Everard Amandine
Laureate of the FNRS BiR&D Multi-Disciplinary Thesis Award Life & Health Science (2015)

THESIS 2015

Geurts Lucie "Impact of the gut microbiota on the adipose tissue-endocannabinoid system: role on the development of obesity and associated inflammation". Director: Patrice D. Cani

Leclercq Sophie "Role of the gut micriobiota and inflammation in the physiological control of psychological disorders in alcoholic patients upon withdrawal". Director: Nathalie Delzenne

THESIS In progress

Catry Emilie "Role of the gut microbiota in the control of hepatic cholesterol metabolism by nutrients and hypolipidemic drugs" Director: Nathalie Delzenne

Clara Depommier "Evaluation of the impact of Akkermansia muciniphila on the metabolic syndrome: pre-clinical and clinical investigations". Director: Patrice D. Cani.

Plovier Hubert "Role of intestinal NAPE-PLD on energy homeostasis, glucose metabolism and gut microbiota on the development of obesity and type 2 diabetes" Director: Patrice D. Cani.

Suriano Francesco "Impact of bran-derived prebiotics on host health: in vivo and in vitro approaches" Director: Nathalie Delzenne

Sophie Hiel "Interest of native inulin in the modulation of dysbiosis and metabolic alterations: experimental approach and nutritional interventions in humans". Director: Nathalie Delzenne

Biomedical Magnetic Resonance (REMA)

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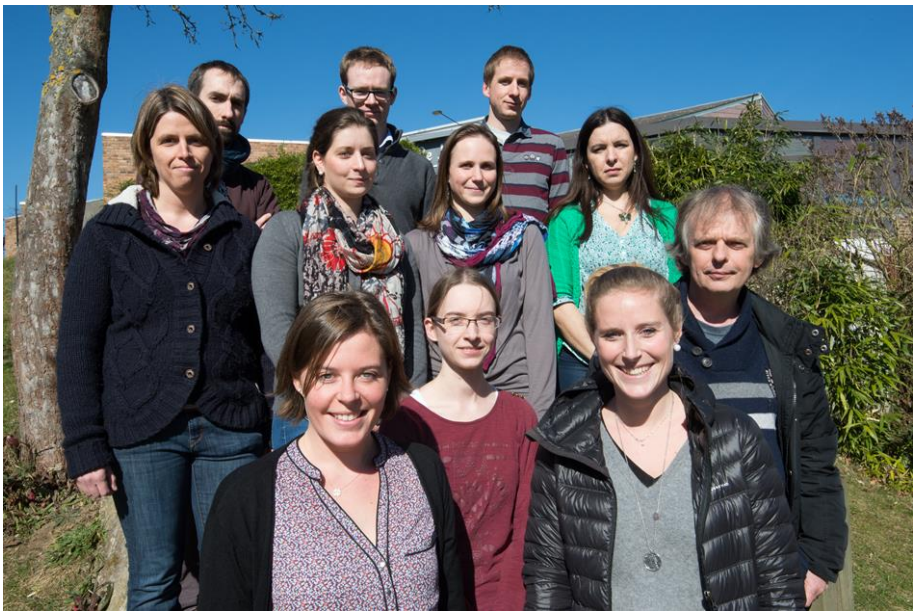

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The goal of this research team is to carry out fundamental and pre-clinical research in biomedical magnetic resonance (NMR or Nuclear Magnetic Resonance and EPR or Electron Paramagnetic Resonance).
The research involves the development of innovative tools using these advanced technologies, and the application of these tools to understand physiology and physiopathology, with a special interest in oncology.
The major theme of the REMA Unit is to understand how the tumor microenvironment influences the response to anti-cancer treatments and to identify early non-invasive markers of tumor response to treatment. For that purpose, three main areas of research involve: (a) the development of tools for monitoring the tumor microenvironment by MR techniques, (b) the application of MR techniques to characterize the tumor microenvironment, and (c) the validation of early non-invasive surrogate markers of tumor response to treatment.

Development of tools for monitoring the tumor microenvironment by MR techniques:

Since several years, we are continuously developing innovative MR technologies to characterize the tumor hemodynamics and its different components: oxygenation of the tissue, perfusion and oxygen delivery, oxygen consumption.

We pioneered developments in EPR oximetry with the characterization of paramagnetic materials possessing favorable features for oximetry. Thanks to these developments, EPR oximetry is routinely used in the laboratory for studying the temporal evolution of tumor pO_2. The technique is unique in a sense that it monitors oxygenation inside a tissue non-invasively and repeatidly from the same site over the time. In a translational approach, we also developed biocompatible forms of these systems. One clinical EPR system (second in the world) has been installed in the laboratory by the end of 2014. This sytem will allow to carry out clinical EPR oximetry studies in oncology and diabetology. We have been also interested in developing new ways to measure oxygen using MRI, namely by using fluorine ^{19}F relaxometry in order to map tumor oxygenation. More recently, we developed and patented a technology called *MOBILE* (Mapping of Oxygen By Imaging Lipid relaxation Enhancement). This technique is based on the change in relaxation of the proton lipids induced by the oxygen which is paramagnetic and acts as an endogenous oxygen sensor. By using special MRI sequences that measure the relaxation of lipids, it is possible to generate parametric maps of the tissue oxygenation. This method is presently under qualification in pre-clinical models and in cancer patients (head and neck cancer, gliomas).

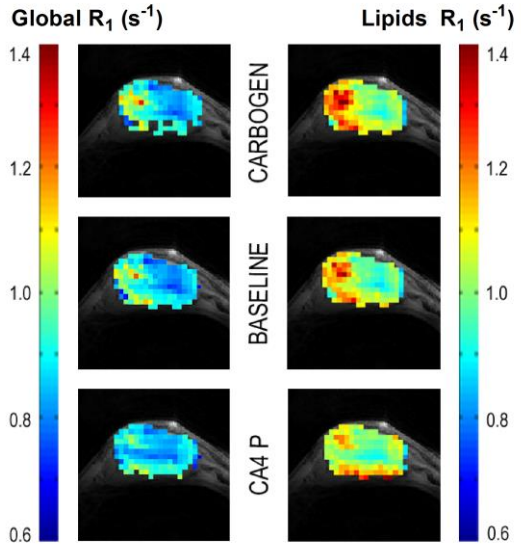


Figure 1. Typical maps of global R_1 and R_1 of lipids obtained on the same mice at baseline, and after hyperoxic and hypoxic challenges performed with carbogen and CA4, respectively. For this tumor, actual values of pO_2 was 6.1 mm Hg at baseline increased to 9.0 mm Hg during the carbogen breathing, and decreased to 5.1 mm Hg 3 hours after CA4 administration.

We are also focusing on the spontaneous fluctuations of tumor oxygenation (tumor acute hypoxia phenomenon or perfusion-limited hypoxia) with new methodologies to provide map of oxygen fluctuations inside the tumors. Regarding hemodynamics, we are characterizing the tumor perfusion and permeability with Dynamic Contrast-Enhanced (DCE) – MRI. On the other hand, we have developed and are continuously developing new methodologies to measure in vivo the tumor oxygen consumption. In more recent studies, we focused on the tumor metabolism which is a target of new therapeutic strategies. More specifically, ongoing studies are focusing on the non-invasive measurement of the extracellular pH, on the characterization of Warburg/oxidative tumor phenotypes and on the link between tumor cell metabolism and cell proliferation.

Besides the applications of MRI in tumors, we are actively developing new applications of in vivo EPR. In this field, we evaluated the capabilities of EPR imaging to provide maps of melanomas. We are also involved in an international collaboration using EPR as a tool in retrospective dosimetry by measuring the free radicals that are generated in teeth and in bones after irradiation. We are also developing new spin traps for measuring mitochondrial superoxide.

Applications of MR (EPR and NMR) to characterize the tumor micro-environment:

One main issue is to characterize how the tumor microenvironment influences the response to therapy. We are testing novel approaches using the modulation of the vascular network and/or the inhibition of the oxygen consumption by tumor cells to increase the response to radiation therapy and/or chemotherapy. Besides the identification of innovative approaches to increase the cytotoxicity for tumor cells, we are trying to define optimal schedule for an optimal therapy.
As illustrative examples of our research activities, we are characterizing the evolution of the tumor microenvironment after therapies that are targeting the tumor metabolism or that are acting in the Choline Kinase pathway. Thanks to the unique tools that have been developed in our laboratory, we are able to understand the evolution of the tumor hemodynamics, and to propose new strategies to optimize radiation therapy and chemotherapy. In another research topic, we are evaluating the influence of the modulation of the tumor hemodynamics on the delivery of anti-cancer agents and on the tumor response.

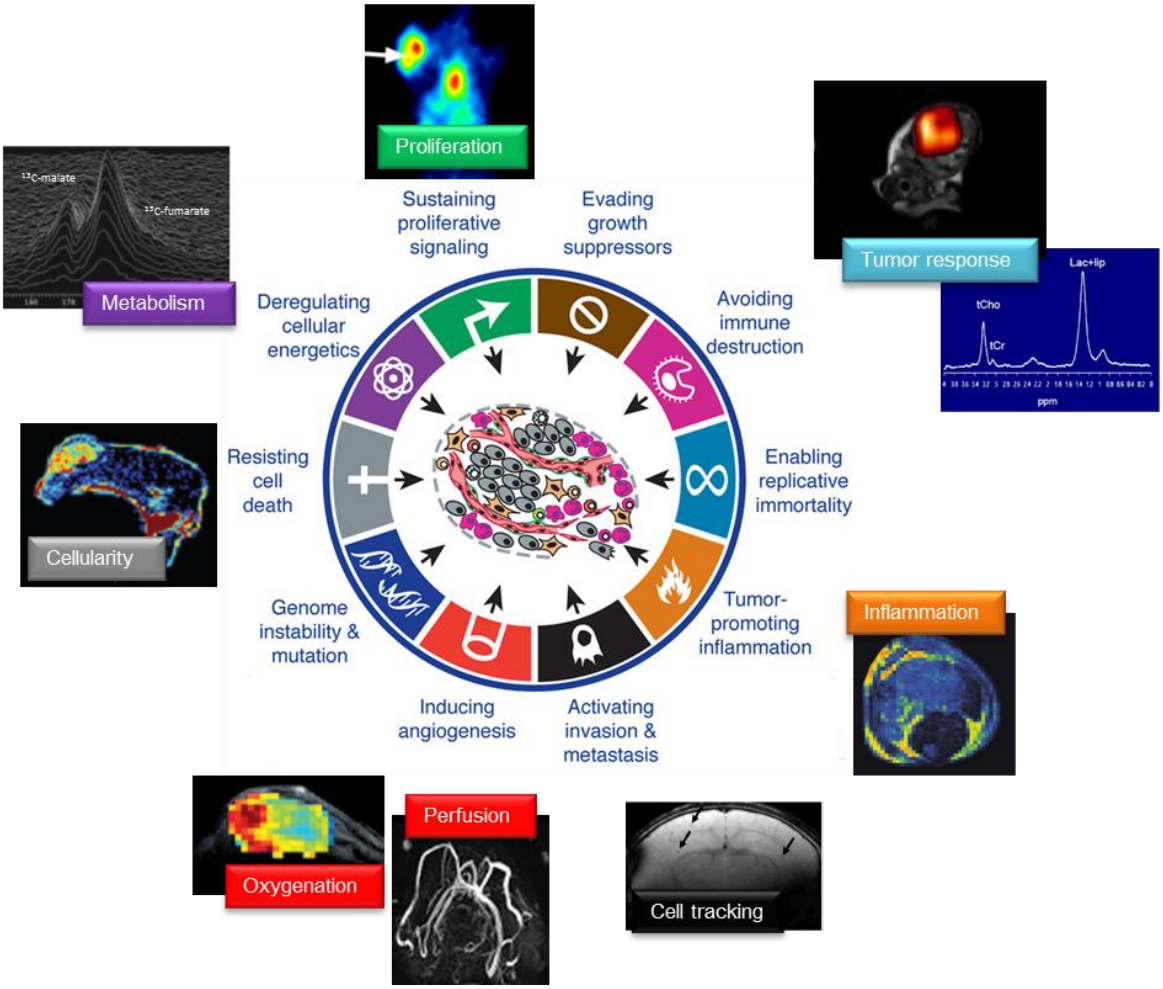
Recent activities in tumor cell labelling by imaging reporters (EPR, MRI, bioluminescence) allowed us to monitor the migration of the tumor cells and their homing in distant organs (monitoring of the metastasis process) and to evaluate determinant factors that influence the metastatic progression (including the role of HIF in the metetastatic progression of breast cancer).

Development of biomarkers predictive of the response to a treatment:

We are currently implementing methods that might be predictive of tumor response early in the treatment regimen and comparing their respective value: use of smart contrast agent directed to cell death, diffusion-weighted MRI (cellularity), spectroscopy of choline (membrane turnover), ^{18}F-FLT PET (cell proliferation). A Dynamic Nuclear Polarization (DNP, "Hypersense") system has been installed in the laboratory and allows the study of

metabolic fluxes using ^{13}C-MRS. We are looking to the value of fumarate/malate and pyruvate/lactate as biomarker of response to anti-cancer treatment. Hyperpolarized substrates are also used for the stratification of tumors that may benefit from innovative therapies that modulate the metabolism of cancer cells. This multi-modal strategy significantly contributes to the identification of early non-invasive imaging markers of tumor response to combined targeted therapies in the transition towards individualized cancer therapy, with a special focus on the resistance to first line therapy in advanced breast cancer, and in advanced melanoma, with the ultimate goal of sparing patient's cycles of futile therapy, and possibly allow them to move to other, possibly experimental therapies. Another illustrative example of our ongoing studies is the use of these imaging biomarkers to evaluate the efficacy of anti-cancer strategies such as dose painting and dose escalation in radiation therapy.

« Imaging hallmarks of cancer »



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Bernard GALLEZ

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Bénédicte JORDAN

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THESIS 2015

Colliez Florence "Monitoring of tumor oxygenation variations using non invasive imaging based on lipid oxygen dependent relaxation in magnetic resonance: pre-clinical and clinical applications" Director: Bénédicte Jordan; Co-Director: Bernard Gallez

Delangre Sébastien "Développement de sequences d'imagerie par resonance magnétique à contraste positif avec des particules d'oxyde de fer super-paramagnétiques" Director: Y. Gossuin ; Co-Director: B. Gallez

Jacobs Damien "Characterization of Wallerian degeneration by in vivo diffusion compartment imaging" Directors: Benoit Macq, Bernard Gallez, Anne des Rieux

Mignion Lionel "Choline as a target and as a marker of tumor response in experimental models: Magnetic Resonance spectroscopy and micro-PET studies" Director: Bénédicte Jordan; Co-Director: Vincent Grégoire

Schleich Nathalie "Multifunctional paclitaxel/SPIO-loaded PLGA-based nano-particles for cancer therapy and magnetic resonance imaging" Director: Véronique Préat; Co-Director: Bernard Gallez

Tran Ly Binh An "Bridging the gap between tumor hypoxia and tumor response. Assessing the value of imaging biomarkers to guide hypoxia-driven interventions in radiotherapy" Director: Bernard Gallez; Co-Director: Vincent Grégoire

THESIS In progress

Acciardo Stefania "Identification of early non-invasive imaging markers of tumor response to BRAF inhibitors in combination with concomitant additional targeted therapy or immunotherapy in melanoma" Director: Bénédicte Jordan; Co-Director: JF Baurain

Cao Pham Thanh Trang "MOBILE as a predictive marker of response to radiation therapy" Director: Bénédicte Jordan; Co-Director: Bernard Gallez

Depreter Géraldine "Effects of inhibition of tumor metabolism on tumor growth and response to treatments" Director: Bernard Gallez; Co-Director: Pierre Sonveaux

Desmet Céline "Influence of the tissue oxygenation on the wound healing in the diabetic foot" Director: Bernard Gallez

Janske Nel "Lipid nanovectors for cancerology" Director: Laurent Lemaire; Co-Director: Bernard Gallez

Kengen Julie "Monitoring tumor redox status by molecular imaging" Director: Bénédicte Jordan; Co-Director: Bernard Gallez

Marchand Valérie "Monitoring of tumor metabolism changes using ultra-high field MRS" Director: Bernard Gallez; Co-Directors: Pierre Sonveaux, Bénédicte Jordan

Neveu Marie-Aline "Warburg Imaging" Director: Bernard Gallez; Co-Director: Olivier Feron

Scheinok Samantha "Spint trapping of the mitochondrial superoxide" Director:

Bernard Gallez; Co-Director: Pierre Sonveaux

Translational Research from Experimental and Clinical Pharmacology to Treatment Optimization (TFAR)

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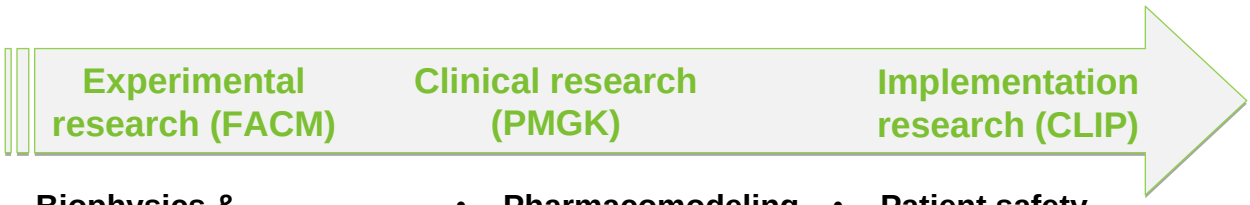

- **Biophysics & molecular pharmacology**
- **In-vitro PK/PD**
- **Pharmacomodeling**
- **Pharmacometrics**
- **Patient safety research, observational & evaluative**

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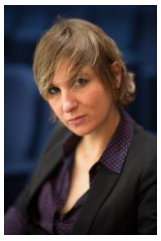

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Pharmacological and clinical evaluation of drugs covers complementary aspects, going from experimental pharmacology to optimization of drug usage in clinical practice via a characterization of patient's specificities that could affect pharmacokinetics or pharmacodynamics.

In this context, we conduct translational research, from bench to bedside, in the field of experimental and clinical pharmacology, with the aim of optimizing drug treatment.

Our common objectives are to use a deep knowledge of the molecular basis of drug action and fate (at both the cellular and the human levels) to achieve personalized pharmacokinetic and pharmacodynamic targets and implement these findings for improving quality of care. Researches focus on high-risk medications (drugs with a narrow therapeutic window or used for severe pathologies) and/or high-risk populations (frail, immunosuppressed, or polymedicated patients).

Within this structure, principal investigators are more specifically experts in one of these three disciplines, with FACM (cellular and molecular pharmacology group; Marie-Paule Mingeot-Leclercq and Françoise Van Bambeke) being mainly oriented towards experimental research, PMGK (integrated pharmacometrics, pharmacogenomics and pharmacokinetic group; Laure Elens) towards clinical research, and CLIP (clinical pharmacy group; Olivia Dalleur and Anne Spinewine), towards implementation. Some activities are therefore unavoidably independent, but there is a clear willingness of cross-fertilization among us, which is operationalized through the organization of common seminars, the co-supervision of translational thesis, and the submission of common grant applications.

Research directions: The main disciplines that are covered include:

(a) in the field of experimental research:

- Biophysics and molecular pharmacology

The objective is to characterize at the molecular level the interaction between drugs and cells or their constituents, in order to unravel the mechanisms responsible for their pharmacological activity or cellular toxicity. Special emphasis is put on drugs:

1.interacting with membrane lipids, considered as novel targets for antibiotics or antitumoral therapies;

2.accumulating within the cells, in order to characterize the molecular cascades leading to cell toxicity.

This fundamental research (mainly performed in FACM) is closely connected to more applied investigations on pharmacokinetics and pharmacodynamics that make use of the evidenced concepts to elaborate and evaluate innovative therapeutic strategies.

- In vitro pharmacokinetics and pharmacodynamics

The objective is to identify and to describe the pharmacokinetic and pharmacodynamic aspects that can affect drug activity, considering both the factors modulating drug bioavailability at the site of action and the influence of the environment on drug activity. More specifically,

- Cellular pharmacokinetic studies examine the accumulation, subcellular distribution, metabolism and active efflux of drugs like antibiotics (FACM) or immuno-suppressants and anticoagulants (PMGK), and explore how specific genetic polymorphisms of cytochromes and efflux transporters can modulate the oxidative

metabolism or the cellular transport of these drugs (PMGK).
- In vitro pharmacodynamic studies in FACM are focused on models of persistent bacterial infections (intracellular survival, biofilms), trying to define the reasons for antibiotic failure and evaluating novel therapeutic strategies.

This research serves as a rational basis for clinical applications to be tested using pharmacometric approaches and may help rationalizing reasons for therapeutic failure or success in the clinics.

(b) in the field of clinical research:

- population pharmacokinetics and pharmacometrics

Pharmacometrics focuses on quantifying variability in pharmacotherapy and considers the complex interaction between genetics, physiology, pharmacology and pharmacokinetics. In our group, this thematic covers multiple fields of complementary expertises essential for the understanding of the fate of xenobiotics administered in humans (in vitro and in vivo pharmacokinetics, pharmacodynamics, population pharmacokinetics, pharmaco-genomics and PK-PD relationships). More specifically, our approaches integrate the study of drug metabolism and active transport as well as modelling and Monte Carlo simulations. Current applications include antibiotics (FACM), immune-suppressants and anticoagulants (PMGK), with the objective of elucidating the determinants of therapeutic responses.
Combining these approaches with experimental research help to elucidate mechanisms underlying clinical findings and facilitates the achievement of new discoveries through explorative investigations.

(c) in the field of implementation research:

- drug optimisation in clinical practice

'Implementation' involves translating results from clinical research into everyday clinical practice and healthcare decision making. It seeks to improve quality of healthcare through the implementation of various interventions. The intent of this research is to understand what, why, and how 'interventions' work in real world settings and to test approaches to optimize them. 'Interventions', in this case, refer to medications and to various processes related to the use of medications and patient safety. Research in clinical pharmacy (CLIP) therefore aims at evaluating the quality of use of medicines in clinical practice, to better understand the determinants of this quality and to evaluate the impact of specific approaches for optimisation on patient safety. Translational research "from bench to bedside and back again" mainly benefits the research work focusing on specific medications such as antibiotics (FACM) and anticoagulants (PMGK), in both the acute care and ambulatory care settings. We use both quantitative research ([quasi]-experimental studies such as randomised controlled trials, cohort studies, surveys,…) and qualitative studies (interviews, focus groups, observations). The approaches for optimisation tested encompass education and training, multidisciplinary teamwork (including working with clinical pharmacists), patient empowerment, audit and feedback, use of protocols,…

Examples of ongoing and projected translational research

At this time, 2 ongoing projects implying two PhD students are illustrating the type of integrative approaches existing between the groups:

- Better defining the positioning of the β-lactam temocillin in our therapeutic arsenal, by evaluating its activity against clinical isolates, and determining its PK profile in specific patient populations in order to propose PK/PD breakpoints and optimal dosages in the clinics based on Monte-Carlo simulations.
- Determining the risk factors for adverse events in patients taking direct oral anticoagulants, including inappropriate use but also individual variations in pharmacokinetics, including those related to genetic polymorphisms.

The next pages present the ongoing projects in each of the groups constituting TFAR.

Cellular and Molecular Pharmacology (TFAR - FACM)

Our team is studying the pharmacology of anti-infective agents (mainly antibiotics) with the aim to decipher the mechanisms responsible for their activity or their cellular toxicity, and to optimize their use in the clinics (based on a better knowledge of their pharmacodynamics and of the risks for selecting resistance). Disciplines and methodologies used involve biophysics, biochemistry, microbiology, cellular and molecular biology, and morphology.

Our main objectives are to decipher, at the molecular and at the cellular levels, the mechanisms of the interaction between anti-infective drugs and

- *bacteria (target cells), with the aim to progress in the understanding of their mode of action and of mechanisms of bacterial resistance;*
- *host cells, with the aim to unravel the mechanism of their transmembrane transport, and to evaluate the consequences of their cellular accumulation for activity and toxicity.*

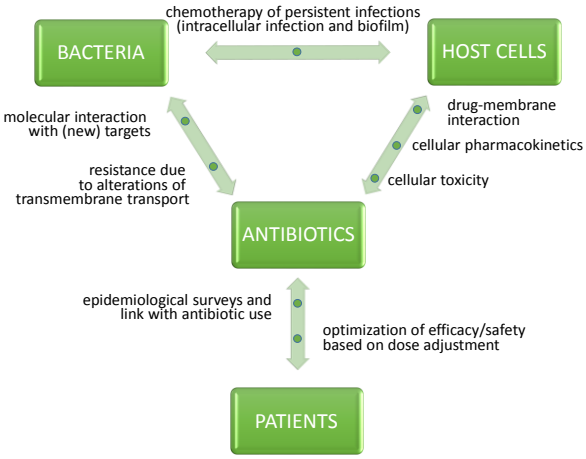
To this effect, we explore

- *at the cellular level, the cellular pharmacokinetics of antibiotics (accumulation, distribution, and efflux in eukaryotic cells), in relation with their activity against intracellular pathogens and with their capacity to cause cellular toxicity (accumulation of lipids; apoptosis).*
- *at the molecular level, the interaction between antibiotics and membrane lipids, the selection of resistance in vitro (with a particular interest for active efflux), and we evaluate antibiotics acting on new, unexploited targets.*

Our experimental approaches include:

- *biophysical approaches aimed at characterizing at the molecular level the interaction between drugs and membrane lipids;*
- *genomic and proteomic approaches aimed at evidencing the effects of drugs on the expression and function of target proteins;*
- *pertinent cellular models for the study of drug pharmacokinetics (accumulation, subcellular distribution, efflux), pharmacodynamics (intracellular infection, biofilm) and cellular toxicity, which are used for exploring the mechanisms governing the interaction between host cells, drugs and bacteria, and to evaluate new molecules or new therapeutic strategies;*

In a broader context, our translational research activities include clinical trials aimed at optimizing antibiotic use (adaptation of their mode of administration or daily dosage) with the aim to increase their efficacy and/or reduce their toxicity (run in coworking with different hospitals in Belgium), and collection of clinical isolates for which we study antibiotic resistance and try to establish a potential link with the treatment received by the patient.



RESEARCH RESULTS

Over the last 5 years, we have published 78 papers, 3/4 of which directed related to our research dealing with anti-infective pharmacology and drug-membrane interactions (1/4 as reviews or book chapters and educational papers related to anti-infective pharmacology or pharmacotherapy, papers in the field of clinical pharmacy).

Our experimental research is oriented in 5 main directions. These are, however, closely linked to one another.

1. Drug-membrane interactions

Biological membranes are fundamental components of all living cells. Their biophysical properties are critical for their numerous functions of mammalian cells including traffic, anchorage of receptors and cell signalling. In this respect, the existence of clusters of proteins and lipids appears as a critical biophysical property of lipid membranes. One of the most often considered models is the raft hypothesis i.e. the partition of lipids between liquid disordered and ordered phases, the latter being enriched in sphingolipids and cholesterol. In prokaryotes also, the existence of lipid domains is now widely accepted with the three main lipids found in bacterial membranes (phosphatidylethanolamine, phosphatidylglycerol and cardiolipin) organized in microdomains.

Our work, focused on the interactions between drugs and lipid membranes, is persued with the aim to provide a more comprehensive and biologically relevant picture of the drug membrane interaction and how the effect of these interactions can modify the biophysical properties of the membranes. Results are put in relation with anticancer and antibacterial activities.

The main questions we address are related to (i) the type of interactions between drugs and lipids, (ii) the modifications of membrane biophysical properties induced by drugs, and (iii) the consequences of these modifications on cellular pharmacokinetics, activity, or toxicity of drugs. Most of these studies are performed by using membrane models (supported bilayers, liposomes [SUVs, LUVs; GUVs]) mimicking (i) eukaryotic and (ii) bacterial membranes. In close collaboration, we developed a range of complementary methods including AFM, ^{31}P NMR, ellipsometry, dynamic light scattering, fluorescence spectroscopy (Laurdan, DPH, DHE, calcein, octadecylrhodamine B…) and confocal microscopy.

(i) Over the three last years, we investigated the ***interaction between lipids of eukaryotic cells and amphiphilic drugs and peptides.***

The aim of this work performed on eukaryotic cells was to investigate the membrane effects induced by amphiphilic pharmacological compounds in relation with their pharmaceutical interests. We focused

on saponins (α-hederin) and lipopeptides (surfactin) both suggested as potential anticancer drugs.

By investigating the molecular mechanism involved in necrosis and apoptosis in leukemic monocytes induced by α-hederin, a monodesmosidic triterpenoid saponin, we demonstrated the critical role of cholesterol. On models of membranes, we showed that α-hederin induced membrane permeabilization by a mechanism which is mainly driven by the formation of saponin/cholesterol domains and the induction of membrane curvature. The latter was dependent on the sugar chain branched on C3 of the aglycone, hederagenin. α-hederin induced phase separation which would be stabilized by a coupling between local composition and monolayer curvature. The lipid phase separation can be observed without any permeabilization of the membrane. Together these results (Figure 1) could be related to the capacity of α-hederin to induce apoptosis in cells and to its potential anticancer effect.

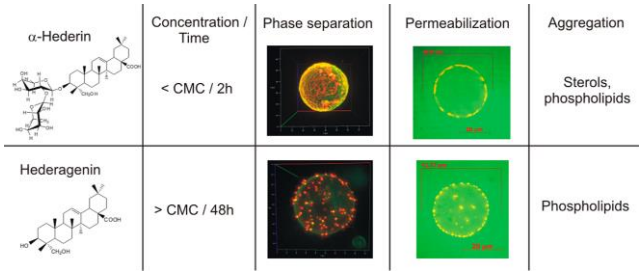


Figure 1. Lorent et al, Langmuir, 2014

Regarding surfactin, a bacterial cyclic amphiphilic lipopeptide known to selectively kill cancer cells, we showed that the presence of rigid domains can play an essential role in the first step of its insertion within the lipid bilayer and its interaction with both the membrane polar heads and the acyl chain region. A mechanism for the surfactin lipid membrane interaction, consisting of three sequential structural and morphological changes has been proposed (Figure 2). At concentrations below the CMC, surfactin inserted at the boundary between gel and fluid lipid domains, inhibited phase separation and stiffened the bilayer without global morphological change of liposomes. At concentrations close to CMC, surfactin solubilized the fluid phospholipid phase and increased the order in the remainder of the lipid bilayer. At higher surfactin concentrations, both the fluid and the rigid bilayer structures were dissolved into mixed micelles and other structures presenting a wide size distribution.

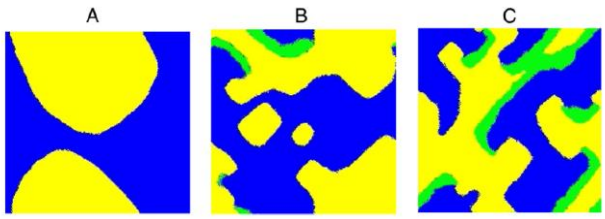


Figure 2. Monolayer grid of 200X200 lipids calculated by the Big Monolayer Method. Each pixel represents a molecule. Blue: DPPC molecule; yellow: DOPC molecule; green: surfactin molecule. (A) DOPC: DPPC at 1:1 molar ratio, (B) DOPC:DPPC:surfactin at 1:1:0.1 molar ratio, (C) DOPC:DPPC/surfactin at 1:1:0.3 molar ratio (Deleu, Lorent et al, Biochem. Biophys. Acta, 2013).

Taking benefit from our expertise, we extended our approaches to **ginsenosides** and to **Budesonide/Cyclodextrins** complexes with the aim to understand how changes of lipid phases could be related to different activities on cell membranes and to pharmacological effects.

(ii) In parallel to studies performed on lipids found in eukaryotic cells, we are also interested in the study of the mode of action of ***new antibiotics acting on bacterial lipid membrane***.

The increased bacterial drug-resistance against traditional antibiotics becomes a major challenge in healthcare and creates an urgent need to develop novel

compounds to treat infectious diseases. Taking benefit from a collaboration with chemists working the Département of Pharmacochimie (the University of Grenoble, France), we explored the interactions of original amphiphilic derivatives of the aminoglycoside neamine on lipids found in membranes of Gram-negative bacteria.

We showed that some derivatives of neamine are active against sensitive and resistant *P. aeruginosa* strains as well as *S. aureus* strains including strains expressing enzymes modifying aminoglycosides, efflux pumps, or rRNA methylases. The mechanism of action is different from inhibition of protein synthesis as observed for conventional aminoglycosides, and results from membrane destabilization.

To decipher, at the molecular level, the mechanism involved in this antimicrobial effect, we determined how the loss of membrane integrity due to LPS binding can affect the integrity of the cytoplasmic leaflet of the outer membrane as well as the integrity of the inner membrane. On *P. aeruginosa*, we monitored membrane permeabilization (NPN and PI assays) and membrane depolarization (DiSC3(5) fluorescence). The interactions of selected amphiphilic aminoglycosides with the three main lipids of the inner membrane of *P. aeruginosa*, PE (phosphatidylethanolamine), PG (phosphatidylglycerol) and CL (cardiolipin) and their effects, is investigated by using relevant membrane models, GUVs (Giant Unilamellar vesicles) for confocal microscopy and lipid monolayers for Langmuir isotherm compression. By confocal microscopy, we are monitoring the lipid domains especially those enriched in cardiolipin.

To enhance the selectivity, we are going to investigate a series of new derivatives varying by the nature of the hydrophobic tail (naphtyl, alkyl, alkyl) as well as the central backbone (neamine versus neosamine) or the position and the number of substitution on the central backbone to define optimal amphiphilicity (Figure 3). This should offer promising prospects in the search for new antibacterials against drug – or biocide – resistant strains.

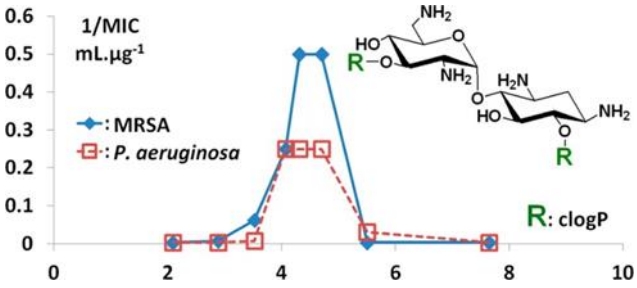


Figure 3. Zimmermann et al, J. Med. Chem. 2013

Our results bring into light fundamental concepts which could be important in membrane-lipid therapy in which the molecular targets are the lipids and the structure they form. The role of lipids can be (i) to facilitate membrane bending and the formation of highly curved intermediates, reducing the energy barriers of fission and fusion and (ii) to recruit specialized proteins. Influencing curvature directly as well as indirectly by targeting negative intrinsic curvature of lipids or in impairing the soft mechanical behavior could be a new approach for antibiotic design.

2. Cellular toxicity of antibiotics

A second area where the interactions between drugs and lipids are critical is **the cross-talks between cellular organelles.** A

better knowledge of these interaction pathways could allow a better knowledge of the molecular mechanism induced by drugs. We focused on the nephrotoxicity induced by aminoglycosides, and particularly gentamicin, by using a combination of biochemical, morphological and biophysical techniques.

At therapeutic concentration, gentamicin accumulates in lysosomes of proximal tubular cells after endocytosis and induces apoptosis via mitochondrial intrinsic pathway, with release of cytochrome c and activation of caspases 9 and 3, but the event cascade between lysosomal accumulation and mitochondria activation remained unclear. In gentamicin-treated LLC-PK1 cells, lysosomal membrane permeabilization, precedes the apoptotic cascade. We clearly evidenced gentamicin-induced lysosomal membrane permeabilization by showing the release to the cytosol of Lucifer yellow, a membrane-impermeant endocytic tracer with a comparable molecular weight as gentamicin, and found by vital confocal imaging that gentamicin induced lysosomal reactive oxygen species (ROS) production prior to acridine orange release from lysosomes and apoptosis. ROS antioxidant or scavenger, catalase and N-acetylcysteine largely prevent these events. We also evidenced the implication of iron in these phenomenon suggested by the protective effect afforded by the iron chelator deferoxamine.

We are further interested in the consequences of lysosomal membrane permeabilization and gentamicin release to the cytosol on the other cellular organelles as proteasome and endoplasmic reticulum (ER), and consequences in terms of apoptosis induction. We assessed chymotrypsin-, trypsin- and caspase-like activities of proteasome in cellular lysates incubated with gentamicin, and showed an inhibition of both trypsin- and caspase-like activities, as an accumulation of ubiquitinated proteins in LLC-PK1 cells incubated with gentamicin. Besides proteasomal inhibition, we evidence p53-pathway implication in gentamicin induced apoptosis, suggested by the protective effect afforded by the p53-inhibitor pifithrin α. As consequence of p53 activation, we evidenced increase in p21 cellular level, phenomenon probably amplified due to proteasome inhibition, also responsible for p27 accumulation and phosphorylation of eIF2α. Cell cycle analysis of LLC-PK1 cells treated with gentamicin tend to show a slight increase in the percentage of cells in phase G2 accompanied by a corresponding decrease in G1 cells. No ER stress, evaluated by GRP78, GRP94 cellular levels and caspase 12 activation, was observed in our conditions.

Finally, to confirm the importance of the role played by cytosolic aminoglycoside in apoptosis induction and nephrotoxicity, we examined whether aminoglycosides from different nephrotoxic potential could be differentiated for apoptosis induction using incubated and electroporated cells.

Studies aiming to decipher the link between the lysosomal permeabilisation, the mitochondrial activation, and the caspase activity leading to apoptosis can be schemed as follows (Figure 4)

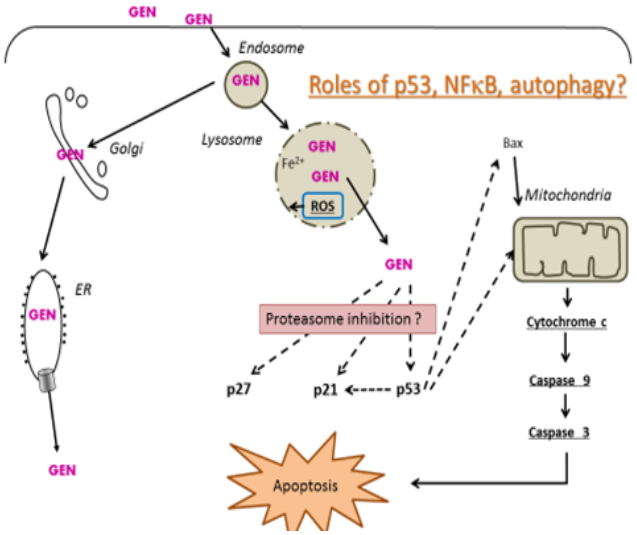


Figure 4

Alterations of membranes of intracellular organelles like lysosomes and mitochondria are therefore at the heart of the apoptotic process induced by aminoglycoside antibiotics. The sub-micrometric domains of membranes of intracellular organelles could also play a critical role in cellular toxicology and in the delicate balance between pro-and anti-apoptotic stimuli.

3. Pharmacokinetics and pharmaco-dynamics of antibiotics in model of persistent infections

Bacterial persistent or recurrent infections are associated with two specific lifestyles, namely intracellular survival and biofilms. We are studying antibiotic activity against these two forms of infections in relationship with antibiotic pharmacokinetics (factors determining antibiotic access to the target).

3.1. Cellular pharmacokinetics

We study the cellular accumulation (including the mechanisms of entry) and the subcellular localization of novel molecules in preclinical and clinical development, as a basis for further studies examining their intracellular activities in specific compartments. We try to decipher the mechanisms for their penetration and distribution within the cells. Over the last years, we have focused our interest on new antibiotic classes, like lipoglycopeptides, ketolides and new oxazolidinones now present on the market. We are now examining innovative antibiotic classes acting on still unexploited targets in order to define their capacity to accumulate within the cells and then to define their interest for the treatment of intracellular infections.

3.2. Cellular pharmacodynamics

In parallel, we study the activity of antibiotics against intracellular bacteria sojourning in different subcellular compartments, mainly *Listeria mono-cytegenes* (cytosol), *Staphylococcus aureus* (phago-lysosomes), and *Pseudomonas aeruginosa.* We developed an in vitro pharmacodynamic approach to compare the efficacy and the potency of the drugs. In brief, we showed that antibiotics are in general less effective but equipotent against intracellular than against extracellular bacteria, irrespective of their accumulation level. We also studied specific phenotypes (Small Colony Variants [SCV] of *S. aureus*) known for their intracellular persistence and poor susceptibility to antibiotics and examined the interest of antibiotic combinations against multiresistant strains. The data generated with these models have been incorporated to the dossier having led to the registration of the last antibiotics brought on the market.

We are now trying to identify specific genes involved in intracellular persistence. Conversely, we are also examining the role of virulence factors (mainly type three secretion systems) in cytotoxicity and are

determining the molecular mechanisms thereof. Specifically, using a collection of clinical isolates from acute infections, we showed that, depending on the type of toxins produced, *P. aeruginosa* caused cell death by necrosis or by pyroptosis consecutive to the activation of the inflammasome cascade. On this basis, we are now studying inhibitors of virulence as adjuctive therapy to antibiotics.

3.3. Antibiotic activity against biofilms

We developed in vitro pharmacodynamic models to evaluate the activity of antibiotics against biofilms made of *S. aureus*, *S. pneumoniae* or *P. aeruginosa*. We showed that antibiotic efficacy and relative potency are considerably reduced in biofilms as compared to planktonic cultures. With *S. aureus*, we found that biofilms made of clinical strains isolated from patients suffering from persistent infections are still more refractory to antibiotics. We could demonstrate that this was mainly due to a default of penetration of the antibiotics within these biofilms, which could attribute to the matrix composition (polysaccharide content) (Figure 5). We are now exploring innovative strategies in order to disrupt this matrix and increase antibiotic activity. With *S. pneumoniae,* we studied clinical isolates from patients suffering from chronic obstructive pulmonary disease. We examined wether bronchodilators taken by these patients could modify biofilm formation and response to antibiotics. We could show that anticholinergic drugs completely disrupted the matrix while beta-agonists stimulated bacterial neuraminidase, an enzyme involved in biofilm remodelling, with as consequence, an increase in antibiotic activity. These data show that bronchodilators effects could thus have favourable effects on the outcome of these patients beyond their known pharmacological activity.

4. Antibiotic efflux and permeability resistance mechanisms

We demonstrated the role of active efflux as a mechanism responsible for the intrinsic resistance of *P. aeruginosa* to specific antibiotics, like temocillin, or macrolides.

For macrolides, we demonstrated also that it regains activity when bacteria are cultivated in clinically-relevant media (serum, broncho-alveolar lavage), because of an increased permeability of the bacterial outer membrane in these specific environments. We are now studying the impact of efflux on resistance to temocillin in an international collection of strains isolated from cystic fibrosis patients. We also took advantage of the existence of this collection with impressive proportion of multiresistant strains for evaluating the impact of a novel inhibitor of beta-lactamase currently in phase III of clinical development on resistance to ceftazidime, one of the first-line drugs in these patients (Figure 6).

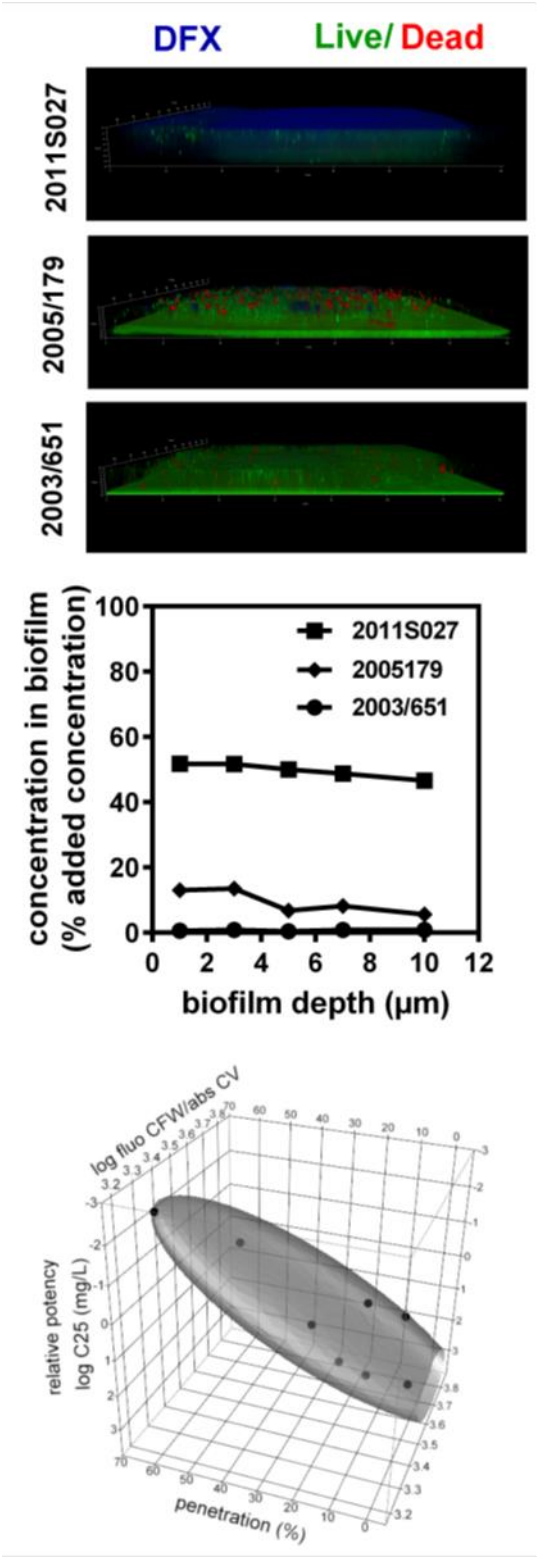


Figure 5. Penetration of the fluoroquinolone delafloxacin in S. aureus biofilms as a predictor of activity.
Top: Confocal images of biofilms from three clinical isolates of S. aureus incubated with delafloxacin [blue], and labelled with live/dead staining [red: dead; green: live].
Middle: The graph compares the relative penetration of the antibiotic within the depth of the corresponding biofilm, expressed in percentage of the added concentration.
Bottom: Correlation between relative potency of delafloxacin, its penetration within biofilms and the proportion of polysaccharides in biofilms, based on data obtained with 8 bacterial strains. Relative potency is estimated as C25 (concentration needed to reduce of 25 % viability within biofilms, penetration within biofilms) is determined in confocal microscopy, and the ratio of calcofluor white [evaluating polysaccharide content] fluorescence to crystal violet absorbance [evaluating biomass] is calculated based on quantitative determinations . The shaded areas show the normal contour density contour.

In parallel, we study the efflux of antibiotics from phagocytic cells and try to identify, at the phenotypic and genotypic levels, the transporter(s) involved. We also study the consequences of this active efflux in terms of cellular toxicity of antibiotics and of modulation of their activity against intracellular bacteria.

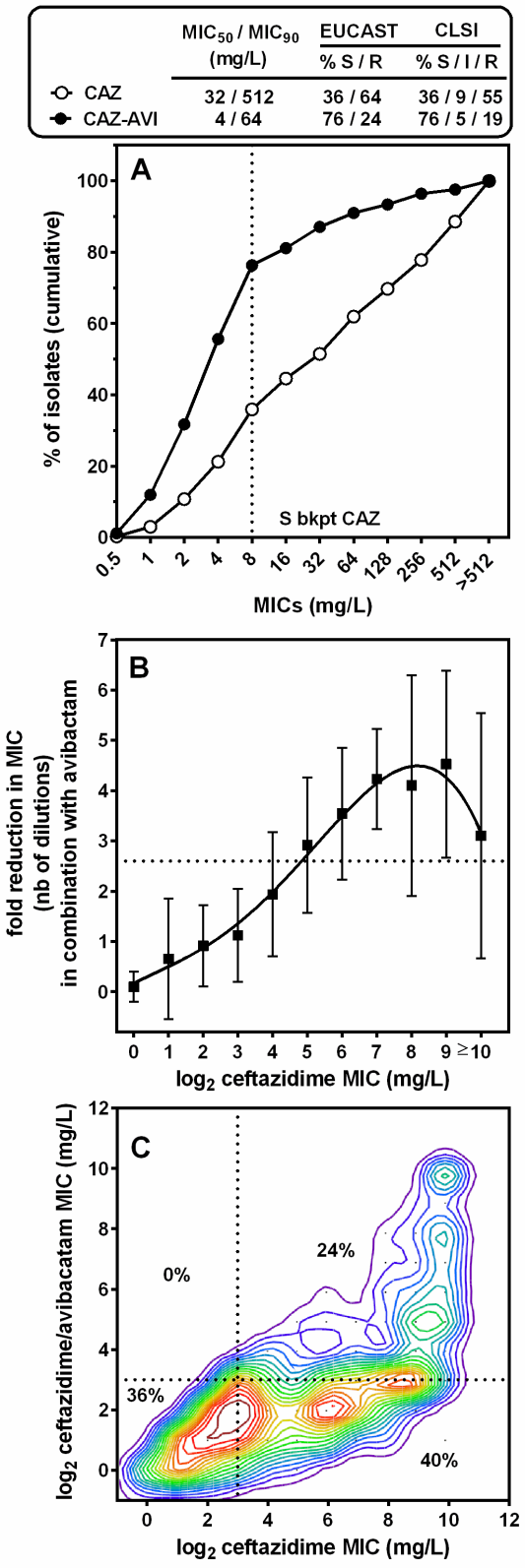


Figure 6: Effect of avibactam (4mg/L) on the activity of ceftazidime against 334 isolates of P. aeruginosa collected from cystic fibrosis patients.
***A:** Cumulative MIC distribution with indication of MIC50, MIC90 and percentage of susceptibility according to the interpretive criteria of EUCAST (S ≤ 8 mg/L; R > 8 mg/L) and CLSI (S ≤ 8 mg/L; R > 16 mg/L). The dotted line points to the limit between susceptible and resistant strains according to EUCAST.*
***B:** Reduction in the MIC (± SD) of ceftazidime (expressed in number of dilutions) when combined to avibactam as a function of the ceftazidime MIC. The data were used to fit a log Gaussian equation (R2 = 0.979) allowing to calculate that the maximal amplitude of change (no. of dilutions; 4.3 ± 0.14) occurred for an MIC of 229 ± 29 mg/L.*
***C:** Correlation between MICs of ceftazidime alone and ceftazidime/avibactam for each individual strain in the collection using quantile density contour analysis. Colours (from warm [red] to cold [blue]) are indicative of the number of strains for each MIC combination. The dotted lines point to the MIC value above which the isolates are considered resistant strains according to EUCAST interpretive criteria and the figures indicate the percentage of strains in each quadrant. Chalhoub et al, 2015*

5. Novel antibiotic targets and drug design

In a world of increasing resistance, discovery of antibiotics acting on new, unexploited targets is an important medical need. In coworking with groups active in pharmaceutical chemistry or in pharmacognosy (within the institute or outside), we evaluate the activity of new compounds and try to decipher their mode of action.

Our clinical research aims at optimizing the scheme of administration of antibiotics in terms of ease of administration, safety, and efficacy, taking into account their pharmacodynamic properties.
At the present time, we are evaluating administration by continuous infusion or prolonged infusion of beta-lactams. In the framework of an ongoing European project, we are evaluating the interest of implementing in the clinics a rapid method

for assaying these drugs in the serum (< 1 h), allowing for immediate dose readaptation taking into account pharmacokinetic variations in intensive care patients. We also perform pharmacokinetic studies in specific patients populations (like haemodialysis patients) in order to propose optimize therapeutic doses. We are also involved in a project financed by the Walloon region aiming at developing a rapid assay for the determination of colistin concentration in serum.

SELECTED PUBLICATIONS
Marie-Paule MINGEOT-LECLERCQ

Sautrey G, Zimmermann L, Deleu M, Delbar A, Souza Machado L, Jeannot K, Van Bambeke F, Buyck JM, Decout JL, Mingeot-Leclercq M-P. New amphiphilic neamine derivatives active against resistant Pseudomonas aeruginosa and their interactions with lipopolysaccharides. Antimicrob Agents Chemother. (2014), 58:4420-4430.

Lorent J., Le Duff C.S., Quetin-Leclercq J., Mingeot-Leclercq M-P. Induction of highly curved structures in relation to membrane permeabilization and budding by the triterpenoid saponins, alpha- and delta-Hederin. Journal of Biological Chemistry (2013), 288: 14000-14017.

Denamur S., Tyteca D., Marchand-Brynaert J., Van Bambeke F., Tulkens P.M., Courtoy P.J., Mingeot-Leclercq M-P. Role of oxidative stress in lysosomal membrane permeabilization and apoptosis induced by gentamicin, an aminoglycoside antibiotic. Free Radical Biology & Medicine (2011), 51: 1656-1665.

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SELECTED PUBLICATIONS
Françoise VAN BAMBEKE

Vandevelde N.M., Tulkens P.M., Muccioli G.G., Van Bambeke F. Modulation of the activity of moxifloxacin and solithromycin in an in vitro pharmacodynamic model of S. pneumoniae naive and induced biofilms. Journal of Antimicrobial Chemotherapy (2015), 70:1713-1726.

Siala W., Mingeot-Leclercq M-P., Tulkens P.M., Hallin M., Denis O., Van Bambeke F. Antibiotic activity against biofilms from Staphylococcus aureus clinical isolates: factors determining the activity of the investigational fluoroquinolone delafloxacin in comparison with daptomycin and vancomycin. Antimicrobial Agents and Chemotherapy (2014), 58: 6385-6398.

Buyck J.M., Tulkens P.M., Van Bambeke F. Pharmacodynamic evaluation of the intracellular activity of antibiotics towards Pseudomonas aeruginosa PAO1 in a model of THP-1 human monocytes. Antimicrobial Agents and Chemotherapy (2013), 57: 2310-2318.

Buyck J.M., Plésiat P., Traore H., Vanderbist F., Tulkens P.M., Van Bambeke F. Increased susceptibility of Pseudomonas aeruginosa to macrolides and ketolides in eukaryotic cell culture media and biological fluids (serum,bronchoalveolar lavage) by decreased expression of OprM (efflux impairment) and increased outer membrane permeability. Clinical Infectious Diseases (2012), 55: 534-542.

Garcia L.G., Lemaire S., Kahl B., Becker K., Proctor R.A., Tulkens P.M., Van Bambeke F. Intracellular forms of menadione-dependent Small-Colony Variants of methicillin-resistant Staphylococcus aureus are hypersusceptible to beta-lactams in a model of THP-1 cells due to cooperation between vacuolar acidic pH and oxidant species. Journal of Antimicrobial Chemotherapy (2012), 67: 2873-2881.

THESIS
In progress

Ameryckx Alice "Design and synthesis of inhibitors of DD-ligases, enzymes participating to the synthesis of peptidoglycan synthesis in the bacterial cytosol" Directors: Raphaël Frederick, Françoise Van Bambeke

Anantharajah Ahalieyah "Intracellular infection by *Pseudomonas aeruginosa*: role of multidrug efflux systems and virulence factors in induction of apoptosis and inflammation and evaluation of new therapeutic strategies" Directors: Françoise Van Bambeke, Marie-Paule Mingeot-Leclercq

Bastos Miranda Ana "Setting and assessing an optimised way of administering beta-lactam antibiotics in severely-infected hospitalised patients" Directors: Françoise Van Bambeke, Anne Spinewine

Catteau Lucy "Search of compounds with antibiotic activity or of inhibitors of resistance mechanisms from natural sources" Directors: Joëlle Quetin-Leclercq, Françoise Van Bambeke

Chalhoub Hussein "Temocillin and Pseudomonas aeruginosa: study of intrinsic and acquired resistance mechanisms" Director: Françoise Van Bambeke

Giro Das Santos Andreia "Drug-Membrane Interactions – A Focus on Nystatin and a Novel Budesonide-Cyclodextrin Complex" Directors: Marie-Paule Mingeot-Leclercq, Liana C., Silva (Medicines and Pharmaceutical Sciences (Universidade de Lisboa)"

Léonard Catherine "interaction of ginsenosides with membrane lipids and

consequences for the activity of transmembrane proteins" Director: Marie-Paule Mingeot-Leclercq

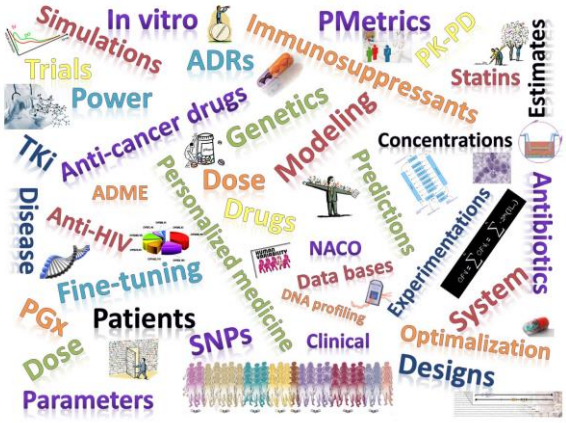
Milosevic Tamara "Cellular pharma-cokinetics and toxicity of oxazolidinones" Director: Françoise Van Bambeke

Mustafa Hariri Muhammad "Development and pharmacological, microbiological and pharmaceutical evaluation of antibiotic combinations for administration as inhaled powders in cystic fibrosis patients" Directors: Françoise Van Bambeke, Francis Vanderbist (SMB laboratories)

Peyrusson Frédéric: "Activity of new antibiotics against intracellular forms of Gram positive bacteria in relation with factors determining intracellular persistence" Director: Françoise Van Bambeke

Integrated PharmacoMetrics, PharmacoGenomics and PharmacoKinetics (TFAR - PMGK)

The PMGK group was created in 2013 with the appointment of L. Elens as a professor in pharmacogenomics and pharmacokinetics. The principal focus of this group is the development and the harmonization of personalized medicine. It mainly aims at characterizing the pharmacokinetic (PK) behavior of drugs in humans using quantitative approaches. The research activities cover multiple fields of expertise such as PK, Pharmacodynamics (PD), Population PK (PPK), Pharmacogenomics (Pgx) and PK-PD relationships, all being essential for the understanding of the fate of xenobiotics administered in humans. More specifically, the PK as well as the Pgx expertise covers in silico, in vitro and in vivo approaches of drug metabolism, all indispensable and complementary to elucidate the determinants of therapeutic responses.



RESEARCH RESULTS

1. Opioids

Painful procedure usually requires analgesia and sedation, especially when it involves new born. Commonly, these therapies implicate the use of opioids. Inter-individual differences in sensitivity to pain and opioid analgesics are well described in adults and might be relevant also in the neonatal population for whom this pain treatment happens during a vulnerable and critical period of central nervous system development. Genetic variants located in genes implicated in the opioid response pathway might explain the various phenotypes related to pain susceptibility and response to opioid therapy. As a result, a multimodal balanced approach aiming at pain relief without the use of unnecessary harmful drugs and/or excessive dosage is recommended but present knowledge appears as rather limited. In the past, our group has achieved some promising advancements in that particular field by showing that two particular genetic variants are likely to predispose preterm newborns to diminished opioid-induced pain relief. In parallel, in another investigation, we have highlighted a significant association between a variant located in a gene involved in the PK pathway of morphine and the production of its metabolites giving new leads to explain individual differences in treatment effectiveness and giving rationale to fine-tune the analgesic therapeutic arsenal with might be inadequate or too aggressive for infants. In 2015, we have confirmed that pharmacogenetics has a role to play in the

management of pain and that we are not all equal regards to pain medication. Indeed, in collaboration with the Erasmus MC (Rotterdam), in newborns, we have shown that *CYP2D6* as well as *OCT1* genotypes contribute independently to the variability observed in the production of the therapeutically active metabolite of tramadol o-desmethyltramadol. This is particularly relevant as, in this frail and developing population, pain medication can be armful if not correctly dosed. Hopefully, if these observations are confirmed, it is possible that *CYP2D6* and *OCT1* genotypes might serve to set up new clinical guidelines in determining the optimal dose for the newborns.

In addition, in collaboration with Lund Children's Hospital (Sweden) and Erasmus MC (Rotterdam), we have investigated how genetics can explain the variability observed in pain relief which was scored on a validated scale after treatment with opioids (Remifentanyl or morphine) among preterm infants needing intubation. We have shown that some infants needed more time to achieve a pain score indicating no pain and this could be partly explain by their genetic make-up. We have pinpoint two genetic variants located in genes involved in the natural modulation of pain (*COMT* and *KCNJ6*).

2. Immunosuppressants used in renal transplantation

In vitro investigations

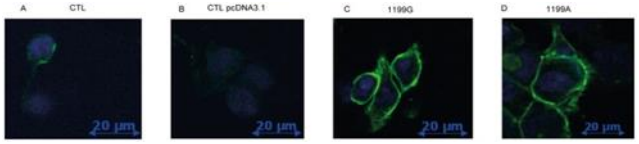


Figure 1: ABCB1 expression analysis by fluorescence microscopy. (A) Untransfected (CTL) HEK293, (B) transfected with empty plasmid (CTL pcDNA3.1) HEK pcDNA3.1 (C) HEK1199G and (D) HEK1199A cells were stained with anti-ABCB1 antibodies (green fluorescence). DAPI was used to stain nuclei (blue).

Our in vitro research activities have allowed unraveling the mechanistic reasons of decreased cellular transport of tacrolimus, an immunosuppressant, in the presence of a particular genetic variant when a transport protein (ABCB1) is overexpressed in a recombinant cell line model. This observation is in agreement with our previously published clinical data. Our study emphasizes thus the importance of *ABCB1* polymorphisms to explain differences in drug response.

Animal studies

In 2013, our group has shown that two SNPs in complete linkage disequilibrium located in the gene of *PPARα* was associated with a higher risk of developing new onset diabetes after transplantation (NODAT), a common toxic effect observed with tacrolimus. In parallele, Patrice D. Cani (MNUT, LDRI) has shown in his lab that knock-out mice for hepatic Myd88 (Myd88 HKO) under high fat diet were characterized by a decreased production of biliary acids production. Previous studies also observed that PPARα deficient mice showed enhanced fatty acid oxidation, decreased glucose degradation and increased insulin resistance. Therefore our hypothesis is that the insulin resistance observed in Myd88 HKO is due to the decreased in PPARα activity and might predispose to diabetes development with Tacrolimus (*figure2*).

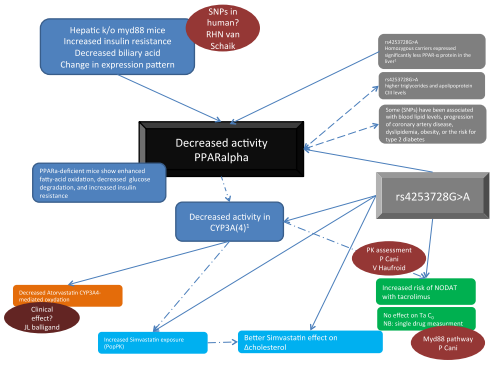


Figure 2: PPARα hypothesis

To test this hypothesis in collaboration with MNUT (Patrice D. Cani) and LTAP (IREC, Vincent Haufroid), we will study the development of insulin resistance in Myd88 HKO after treatment with tacrolimus compared to wild-type controls (*figure 3*). In addition, as PPARα has been also associated with a decreased CYP3A4 activity, we will performed a complete PK analysis in these mice to see the loss of Myd88 activity in the liver and the consequent decreased in PPARα activity has an impact on tacrolimus exposure that might *in fine* explain differences in phenotypes and predisposition to diabetes development.

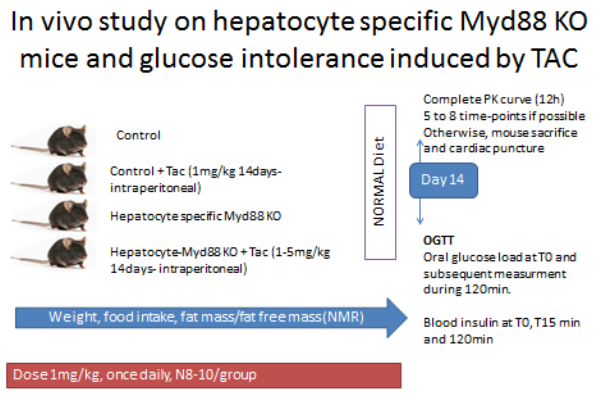


Figure 3: Myd88 HKO tacrolimus protocol study

Human studies

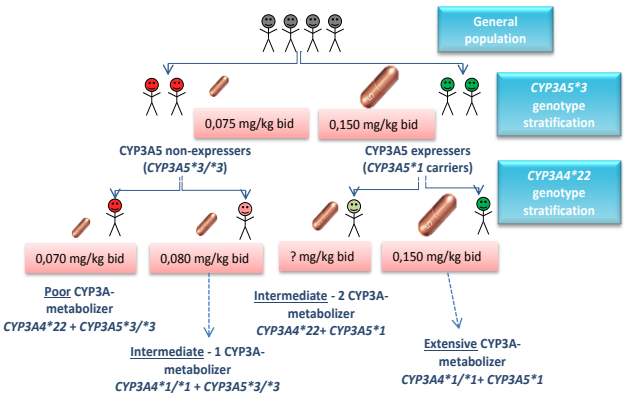


Figure 4: New dosing guidelines for tacrolimus therapy in renal transplant recipients according to CYP3A genotype

Patient survival and graft outcome after kidney transplantation have drastically improved in recent decades, mainly because of major improvements in immuno-suppressive therapy. However, optimal immunosuppression is difficult to achieve in an individual patient, as the majority of immunosuppressive agents are characterized by highly variable PK and a narrow therapeutic window. The use of immunosuppressive drugs is further complicated by their high toxicity profile. The susceptibility to develop adverse events or experiencing therapeutic failure varies strongly between individuals. An important part of this variability in drug response is thought to be the consequence of substantial inter-individual differences in drug metabolism. Some patients have relatively fast drug clearance, while others exhibit a slower drug elimination rate. This variation in drug clearance is of importance, since it might be related to an increased risk of under- or overexposure, which can ultimately lead to a higher frequency of acute graft rejection or adverse events. A collaboration with the Erasmus MC (Rotterdam, The Nethelands) and the KUL (Louvain, Belgium) has permitted to highlight that carriership of genetic variants in the Cytochrome P450-mediated drug metabolism is associated with a rough 30% reduction in *in vivo* metabolic activity. In addition, it was demonstrated that immunosuppressant steady-state drug clearance was equally decreased with the presence of this genetic variant, leading to 50% lower dose requirements. In addition to genetic differences, drug interaction can also impact on therapy outcome. In a hepatic pediatric transplant cohort, we have set up a popPK model to estimate the drug clearance and to decipher how individual characteristics explain the inter-patient clearance variability. Not only had we confirmed that patient genotypes correlate

with drug metabolism but also that concomitant use of CYP3A4 inhibitors impact on drug exposure. All those important discoveries have led our group to propose new dosage guidelines (see figure 4 above) that can be useful in the frame of pre-emptive genotyping and dosage adjustment prior to transplantation, before the initiation of immunosuppressive therapy. In theory, this would lead to a reduced risk of under or over-exposure to the drug in every patient and *in fine*, to decreased risk of undesirable therapy outcome. In a more recent study, we also found that the presence of this Cytochrome P450 genetic variant was associated with the susceptibility of developing cancer with long term immunosuppressive drug treatment, emphasizing the importance of our discovery and that it appears as really imperative to take genetic predisposition into account regards to therapy outcome.

In 2016, our new hypothesis with the implication of PPARα in the clinical response with tacrolimus (see above, animal studies and figure 2), we will work in collaboration with Erasmus MC (Rotterdam) to see if Myd88 genotypes have an impact on the risk of developing NODAT. To that purpose, a total of 101 patients from the IMPACT study have been followed after transplantationand the need of antidiabetic medication after start of therapy has been recorded and is assumed to reflect the development of NODAT. To date, we have selected 5 SNPs in MYD88 that we will screen in the entire population. They all have a minor allelic frequency of >5% and at least one citation in Pubmed with a potential clinical impact.

3. Statins / lipid lowering medication

Since cardiovascular diseases are a real public health problem, lipid lowering medication are widely used to decrease cholesterol and triglyceride levels in the general population. At present, it is estimated that approximately 200 million people are using statins worldwide. There is, however, a great interindividual variation in response to therapy which remains ununderstood and is not mastered. It is supposed that a part of this variability might be attributed to genetic factors. In 2014, we have conducted multiple studies in collaboration with the University of Athens (Greece) that allowed us demonstrating the importance of genetic polymorphisms in the metabolic pathway to explain difference in drug response. As a substantial number of patients are treated with that class of drugs, this could be of unprecedented importance as individualization of therapy can be of tangible health as well as cost benefits.

To pursue these investigations a step further, the group is now collaborating with Prof. Dr JL Balligand (FATH, IREC) to unravel the mystery of the pharmacogenetics of statins. In this context, a PhD student will join the PMGK team in September 2016. We will also work with Prof Giulio Muccioli (BPBL) for the analytical part of the project and with Prof Vincent Haufroid (LTAP, IREC) for the translational aspect. The main observation arising from our previous studies is the influence of the *CYP3A4*22* allele on the lipid lowering response to Simvastatin. This highlights a potential important application of pharmacogenetics for statin therapy but is still in its infancy. Our collaborative project will try to decipher the potential of this promising observation but also to quantify and better characterize the importance of the patient genetic background in explaining differences in the statin response. In that way, the project will take several orientations (figure 5 and 6):

1. The evaluation of the true pharmacokinetic impact of the *CYP3A4*22* allele on the exposure to the drug.

2. The estimation of the incidence of ADR in *CYP3A4*22* carriers and other rare defective allele carriers (e.g. *CYP3A4*20*, *SLCO1B1*5*).
3. Evaluation of the potential added value of taking into account other genetic variations.
4. In vitro assessment of the mechanism of observed associations.
5. In vivo animal studies characterizing the distribution of the drug while lightening on or repressing the expression of proteins.

Figure 5: Workflow of the clinical study

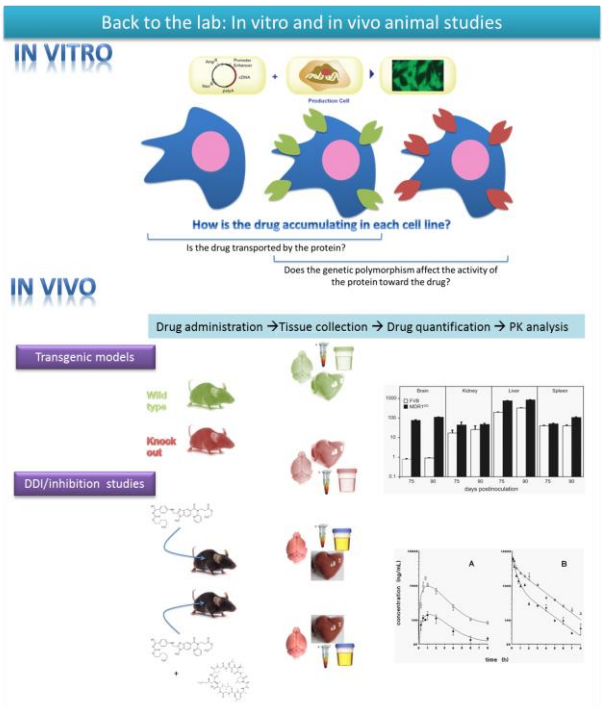


Figure 6: Workflow of the in vitro and in vivo animal studies

4. Anti-HIV drugs

In close collaboration with the infectious disease unit of Saint Luc hospital (Brussels, Belgium), our challenge is to try to better characterize the origins of the large inter-individual variability in response to anti-HIV drugs, focusing on new promising anti-HIV drug treatments. At this moment, we have recruited more than 150 patients treated with a new protease inhibitor, Darunavir, for which literature data are very limited. For that particular project, not only plasma drug concentrations are being monitored in every patient and DNA collected but also intra-lymphocytic concentrations are being determined. To our knowledge, this study constitutes the first large study allowing studying pharmacogenetic determinants of plasma as well as intracellular accumulation of the drug. This is particularly important as lymphocytes represent the site where the drug exerts his therapeutic action. Thus, while variations in plasma drug

concentrations are important to explicate the apparition of adverse drug reactions, differences intracellular concentrations would appear to better correlate with therapeutic efficacy and apparition of virus mutation and/or resistances against the drug. At this stage, the development and the validation of appropriate analytical methods have been completed. Concerning the pharmacogenetic part of the study, we have highlighted that a particular SNP in CYP3A5, an enzyme involved in the metabolism of Darunavir, might predisposed the patient to drug-drug interaction with etravirin that is commonly co-administrated with Darunavir. It appears clear that our observation could be of clinical relevance as it can be predictive of the treatment response. This information will be of crucial importance in the frame of individualized medicine and could be of direct benefit for the patients. Our next objective is to confirm this observation in healthy volunteers by performing a complete PK course to characterize more precisely the real genetic impact of CYP3A5 variations and to quantify the effect for potential dosage adjustments.
In parallel, we are collecting random plasma samples in patients at the "clinique universitaire Saint-Luc" in an aim of performing Darunavir Population pharmacokinetics. At this stage, we have collected approximately 30 samples with a final objective of more than 100 samples. This will be conducted in collaboration with Dr Pierre Marquet (Limoges).

RESEARCH PROJECTS AND RESEARCH STRATEGIES FOR THE NEXT FIVE YEARS

In addition to these ongoing projects, this new group wishes to develop new research axes over the next years, which are briefly explained hereunder.

Clinical/in vivo projects

New anticoagulants

In association with CLIP, we will investigate the PK, PD and Pgx of New anticoagulants (NACO project), mainly Rivaroxaban and Dabigatran in patients with atrial fibrillation hospitalized at Mont-Godinne CHU. For NACO treated patients, PK measurements, anticoagulation efficiency as well as ADRs are being recorded. Additionally to the CLIP project, a Pgx substudy will be included in the protocol. Therapeutic outcomes will be then confronted to the patient genotype and compared among different groups. This study is aiming at identifying high risk patient and potentially to offer a more personalized posology and/or drug therapy.

Pharmacogenetic clinical value and implementation

Eu-PIC www.eu-pic.net HORIZON2020 *European Pharmacogenetics Implementation Consortium*

As clearly demonstrated above, pharmacogenetics has the potential to respond to the increasing burden of chronic disease and the complexity of co-morbidities but also to contribute to the sustainability of health and care systems. If this potential is to be realized at a larger scale, it must first be demonstrated in terms of maintainable benefits, and as a new model of care organization. Demonstration is however complicated by the diversity of European Union health systems. Our group is now involved in the European Pharmacogenetics Implementation Consortium (Eu-PIC, www.eu-pic.net). The consortium aims at providing evidence on methods of pharmacogenetic implementation and benefits of reform while ensuring safety, equity and cost effectiveness. For such a challenge, appropriate measures for

knowledge transfer and capacity building should be defined and set up. The final task will be to produce evidence for a validated model of organization of care based on the concept of personalized medicine through pre-emptive pharmacogenetic strategies and to use policy and decision makers to improve healthcare systems on the basis of quantitative and qualitative indicators. This collaboration englobes the participation of 17 European countries and more than 50 principal investigators.

In vitro projects

Antibiotics

In eukaryotic cells, fluoroquinolone antibiotics are subjected to active transport mediated by proteins belonging to the ATP binding-cassette (ABC) superfamily. The principal aim of this prospected work to be done in close collaboration with FACM is to evaluate in vitro if, and to which extent, constitutional *ABC* genetic variations are likely to influence the intracellular PK of this class of drugs and by consequence, their activity against intracellular bacterial infection.

Tyrosine kinase inhibitors

As for antibiotics, tyrosine kinase inhibitors are also transported by ABC transporters that are subjected to polymorphic activities. As mentioned above, we have previously identified some SNPs in *ABCB1* influencing its in vitro transport activity towards several substrates. More specifically, our current project is investigating how these functional SNPs impact on inhibitors intracellular pharmacology by using recombinant models overexpressing the wild-type ABCB1 or its mutated counterpart by making use of the models developed in our lab.

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Clinical Pharmacy (TFAR - CLIP)

Our research group was created in 2010 by Anne Spinewine who had been appointed as a professor in clinical pharmacy. A second group leader – Olivia Dalleur – was appointed in September 2014. Her previous postdoctoral placement at Harvard Medical School to work on patient and medication safety will further support expanding the research group and broadening the expertise. We therefore hope to elaborate a sound research group strategy, to reinforce leadership, facilitate development, collaborations and search for funds.

RESEARCH THEMES AND OBJECTIVES

Our research focuses on the evaluation of the quality of use of medicines in clinical practice, mainly in populations at risk of adverse drug events, such as older people, patients in intensive care, patients transiting across different settings of care and patient taking oral anticoagulants.

We study the quality of use of medicines in different populations and practice settings, with the following objectives:

- to characterise this quality with regard to prescription, administration, follow-up and continuity of care;
- to understand the factors underlying suboptimal practice;
- to evaluate the consequences of suboptimal practice on clinical (eg adverse drug events, readmission to hospital), economic (cost and indirect costs), and humanistic (eg patient satisfaction) outcomes;
- to evaluate the effect of various approaches for optimisation, such as: collaborative care, health information technology, use of protocols, patient engagement,...

To this effect, we use quantitative as well as qualitative research methods, and we:

- develop and/or validate instruments and tools to measure the quality of use of medicines
- perform qualitative studies and/or surveys to identify the determinants of suboptimal practice
- design, implement and evaluate various approaches for optimisation, that address the causes of suboptimal

practice. Evaluation usually involves using (quasi)-experimental designs, continuous quality improvement studies and observational studies. .

- conduct systematic reviews on the effect of approaches for optimisation

RESEARCH RESULTS AND PERSPECTIVES

In 2015 our group has continued working around 4 main themes:

- Appropriateness of use of medicines in older patients:

- In collaboration with KULeuven (Prof V Foulon), we are coordinating a multicentric study funded by the national health insurance (INAMI). The COME-ON (Collaborative approach to Optimise MEdication use for Older people in Nursing homes) study aims to evaluate the effect of a complex intervention involving inter-disciplinary collaboration on the appropriateness of use of medicines in Belgian nursing homes. In 2015 the research protocol has been finalised, patient recruitment was finalised, baseline data were collected and followed by implementation of the different components of the intervention (education and training, local concertation and multidisciplinary case conferences).
- STOPP (Screening Tool of Older Person's Prescriptions) and START (Screening Tool to Alert Doctors to Right Treatment) criteria aim at detecting potentially inappropriate prescribing in older people. In the context of the COME-ON study, we have developed and validated an algorithm to automatically detect STOPP and START criteria, as well as the 2015 AGS criteria, from our research database.
- We developed a validated French translation of the STOPP and START criteria in collaboration nine French-speaking experts – geriatricians, pharmacologists and a general physician – from four countries (France, Belgium, Switzerland, and Canada). The validation was completed by an inter-rater reliability (IRR) analysis of the STOPP/START.v2 criteria applied to 10 standardized clinical vignettes. We are also evaluating the clinical importance of the criteria in clinical practice.
- A specific case of frequent inappropriate use of medicines in older patients is the underse of anticoagulation for the prevention of cardio-embolism in patients with atrial fibrillation (AF). We aimed at describing and identifying characteristics of anticoagulation among geriatric patients since the introduction of Direct oral anticoagulants (DOACs) through a cross-sectional study of 275 consecutive geriatric patients aged ≥75 years, with AF and clear anticoagulation indication. Underuse of anticoagulation was found in 29% of patients, which is significantly lower than before the marketing of DOACs. We found that older age and aspirin use were the main characteristics associated with anticoagulation underuse.
- The OPERAM project is a European project (H2020) led by University of Bern and start in May 2015. As leaders of WP4 on "clinical outcomes and patient perspective", we have started developing (a) a core outcome set relative to medication review in older people and (b) a method to adjudicate drug-related admissions in older people. We also contribute to the preparation of the protocol for the multicentric controlled trial that will be the core of this European project.

- Sedation and analgesia in intensive care

- Appropriate management of analgo-sedation in the intensive care unit (ICU) is associated with improved patient outcomes. We finalized the analysis of a national survey on current practices and barriers impairing adherence to recommandations. We found

that current analog-sedation practices leave room for improvement. Physicians and nurses meet different challenges in using appropriate analgo-sedation strategies. In a multivariate analysis, we found that workload considerations hamper utilization of sedation scales. Poor familiarity, for nurses, and negative perception of impact on patients'comfort, for physicians, both reduce DSI utilization. Targeting these obstacles is essential while designing quality improvement strategies to minimize sedative use.

- Seamless care with regard to medications:

- The SEAMPAT project is a 3-year project (2014-2017) that aims to develop and evaluate (a) an electronic tool to be used by patients to enter their medication history and (b) an electronic medication reconciliation tool that will facilitate the identification and resolution of medication discrepancies. We lead the project and work in collaboration with 3 other research groups with various expertise including ethics, sociology and IT. We finalised a systematic review on the implementation and effect of electronic medication reconciliation. We also performed focus groups to explore the perspective of patients and healthcare professionals. The evaluation of the first prototypes of the patient application has started and will continue in 2016.

- Quality of use of direct oral anti-coagulants

Direct oral anticoagulants (DOACs) have been developed to address some of the drawbacks of vitamin-K antagonists. However, special attention should be given when using these drugs, especially in patients with renal insufficiency, questionable compliance, and those at high risk of bleeding.

- We started a prospective study to evaluate the prevalence of medication errors with DOACS and explore the causes. In parallel, we continued our collaboration with the Namur Thrombosis and Hemostasis center around the development and validation of new biological tests.

In addition, a project on the monitoring of β-lactams in severely infected patients is run in collaboration with the research group on cellular and molecular pharmacology (main collaborators: A Bastos, F Van Bambeke, P Tulkens).
In 2016 we also plan to pursue collaboration with the Cliniques universitaires Saint-Luc and Harvard Medical School on research about the use of clinical decision support and other health information technology to improve quality and safety of patient care regarding medication use.

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THESIS
2015

Sneyers Barbara: "Use of guidelines by healthcare professionals in the intensive care: easier said than done? The example of analgesia and sedation" Directors: Anne Spinewine, PF. Laterre

THESIS
In progress

Anrys Pauline: "Improving the use of medications for older people in nursing homes" Directors: Anne Spinewine, Veerle Foulon

Bastos Miranda Ana: "Setting and assessing an optimised way of administering beta-lactam antibiotics in severely infected hospitalised patients" Directors: Françoise Van Bambeke, Anne Spinewine

Marien Sophie: "Improving continuity of treatments through patient empowerment and electronic medication reconciliation" Directors: Anne Spinewine, Benoît Boland

Sennesael Anne-Laure: "Optimising the use of direct oral anticoagulants in daily clinical practice: from bench to bedside and back again" Directors: Anne Spinewine, Jean-Michel Dogné

Thevelin Stefanie: "Medication review to prevent avoidable hospital admissions in the multimorbid elderly" Directors: Olivia Dalleur, Anne Spinewine

TECHNOLOGY PLATFORMS

1) MASSMET PLATFORM

The MASSMET platform is an analytical platform applying mass spectrometry analysis to small metabolites and to compounds of biological or pharmaceutical interest.
It provides a support in analytical chemistry mainly through the development of chromatographic methods coupled to mass spectrometry detection, with a particular focus on the detection, identification and quantification of "small molecules" in complex matrices. As such, the expertise provided by the platform is important for numerous labs within the LDRI and the "Health Sector", as well as for labs of the "Sciences and Technology Sector".
To this aim, we share the use of several analytical equipments located both in Brussels (mainly at the LDRI) and at Louvain-la-Neuve (mainly at the ISV). These equipments include (but are not limited to):

- ThermoScientific LTQ – ORBITRAP –XL high resolution mass spectrometer (shown on the right)
- ThermoScientific Trace GC-MS
- Thermoscientific LCQ Advantage mass spectrometer
- ThermoScientific DSQ GC mass spectrometer
- Several chromatographic systems (HPLC, UPLC, GC) using UV, DAD, or FID detectors are also available.

The interest and importance of the expertise of the MASSMET platform is shown by the numerous publications (published and in preparation) that benefited from the data obtained using the equipment and/or expertise of the platform. Examples of such studies involving LDRI research groups include the quantification of antibiotics from cell cultures (TFAR-FACM), the quantification of transcellular transport (ADDB), the quantification of endogenous metabolites from microorganisms, cells, tissues (BPBL – MNUT – TFAR-FACM), the identification of metabolites from plants (GNOS), the quantification of endogenous and exogenous metabolites in plasma (BPBL – ADDB – GNOS) and the determination of the nature and purity of compounds of synthetic origin (CMFA). An exhaustive list of collaborations (within and outside the LDRI) and publications is available on the platform website (http://www.uclouvain.be/en-massmet.html).

2) NUCLEAR & ELECTRONIC SPIN TECHNOLOGIES (NEST) PLATFORM

The NEST platform accommodates cutting-edge MR technologies (Magnetic Resonance Imaging or MRI, Electron Paramagnetic Resonance or EPR, and Dynamic Nuclear Polarization or DNP) dedicated to studies on biological samples and on small animals. These technologies provide convenient biomarkers for monitoring (patho) physiological parameters and the response to pharmacological treatments. The platform provides a support and expertise in the application of magnetic resonance (MRI, MRS, EPR) in pharmaceutical and biomedical sciences.

Equipments

Magnetic Resonance Imaging

Horizontal High-Field NMR Sytem (Bruker Biospec 11.7/16) operating at 11.7 Tesla for MRI and MRS on small animals (bore diameter of 16 cm).
This system is equipped with:

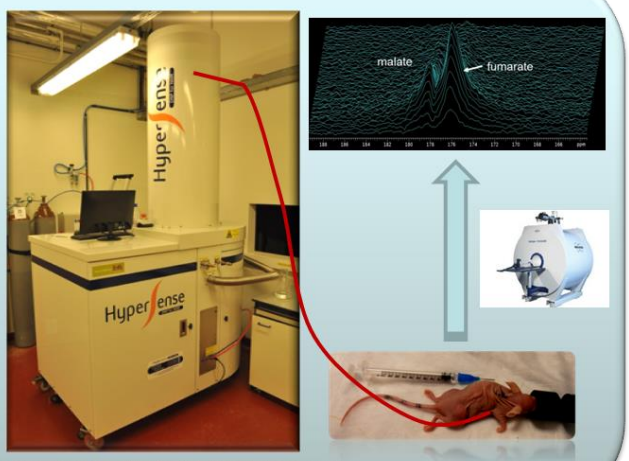
- ❑ ^{1}H-NMR:
  - ✓ cryoprobe
  - ✓ whole-body coil (mouse, rat)
  - ✓ phase-array coil (4 channels, heart studies)
  - ✓ ^{1}H-^{13}C surface coil
- ❑ ^{1}H-^{31}P surface coil
- ❑ ^{1}H-^{17}O surface coil
- ❑ ^{1}H-^{19}F surface coil

Bruker Biospec 11.7/16

Dynamic Nuclear Polarization

- ❑ Hypersense system able to hyperpolarize ^{13}C-enriched substrates for in vivo monitoring of metabolic fluxes:
  - ✓ fumarate/malate
  - ✓ pyruvate/lactate

This system is used in combination with the MRI system for detections of the metabolites using ^{13}C-MRS coils.

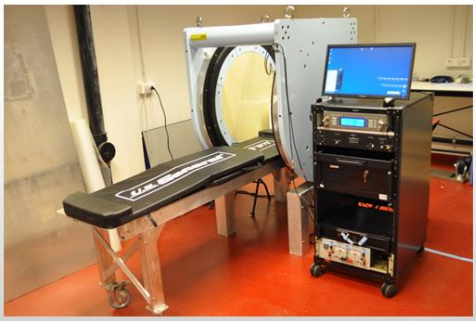


Hypersense, Oxford Instruments

Electron Paramagnetic Resonance

- Preclinical EPR:
  - ✓ EPR Spectrometer 9 GHz (Bruker EMX) for in vitro studies
  - ✓ EPR Spectrometer 9 GHz (Magnettech Miniscope MS200) for in vitro studies
  - ✓ EPR Spectrometer 1 GHz for in vivo studies Magnettech
  - ✓ EPR Imaging and spectroscopy 1 GHz / 9 GHz (Bruker Elexsys) for in vitro and in vivo studies

- Clinical EPR:
  - ✓ Clin-EPR Spectrometer 1 GHz
  - ✓ 2nd system worlwide
  - ✓ Human superficial tissue oxygenation measurements (diabetic foot, head & neck tumors,…)



Support to research activities

Pre-clinical MRI and MRS	EPR spectroscopy and imaging
• In vivo anatomical structures • Metabolism (NMR spectroscopy) • Vessels architecture (micro-angiography) • Tissue perfusion (by Dynamic Contrast Enhanced MRI, *DCE-MRI*) • Oxygen and pH measurements • Heart physiology (ventricle function) • Cell death (Microscopic water diffusion) • MRI-based cell tracking • Assessment of drug delivery • Fiber tracking	• Free radicals measurements • Free radicals characterization by spin trapping • Quantification of melanin / melanoma cells in tissues • Molecular dynamics, microviscosity, micropolarity in tissues and drug delivery systems • Dosimetry (retrospective dosimetry in bones and teeth, dosimetry in phantoms • Tissue oxygenation and Oxygen consumption • Redox status, pH

The utility and importance of the expertise of the Pre-clinical MR platform is testified by the numerous publications that benefited from the data obtained using the equipment and expertise of the platform. Illustrative examples involving LDRI or Health Sector research groups include the characterization of new drug delivery systems (ADDB/LDRI), characterization of spinal cord regeneration (ADDB/LDRI), identification of free radicals involved in toxicological processes (MNUT,MORF/LDRI,IREC), characterization of the tumor microenvironment (REMA/LDRI),

characterization of dental resins (ADDB/LDRI), characterization of angiogenic process and tumor metabolism (FATH/IREC), oxygenation of pancreas islets grafts (CHEX/IREC), ovarian grafts (GYNE/IREC), endometrium grafts (CELL/DDUV), cardiac function (FATH, CARD/IREC), validation of PET tracers (MIRO/IREC).

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